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Isolation of borneol and bornyl acetate from *Ferulago macrocarpa* by microwave irradiation

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Microwave- assisted extraction (MAE), Solvent-free microwave extraction (SFME), Microwave-assisted hydro-distillation (MAHD), Hydro-distillation (HD), Microwave irradiation (MW).

ABSTRACT

Background & Aim: A simple and rapid microwave- assisted extraction (MAE) procedure was developed and optimized for the isolation of borneol and bornyl acetate from *Ferulago macrocarpa*. *Ferulago macrocarpa* (Apiaceae) is an endemic plant in Iran.
Experimental: Dried powdered aerial parts of plants were soaked in water and irradiated with microwave in a Clevenger extraction approach under optimum conditions (520 W and 0.5 h).
Results & Discussion: Compared to traditional heating methods by Clevenger, the present method is more efficiency and proceeds in a short time. The isolation rates of borneol and bornyl acetate increased by about 2 and 1.3, respectively, as compared to the conventional thermal methods. Low cost, easy extraction, high purity of the extracted products and high efficiency are the advantages of this method. Furthermore, solvent free extraction of the essential oils is a good method for the development of green chemistry.
Recommended applications/industries: The essential oil of this plant obtained by MAHD and HD methods, contained bornyl acetate as the most abundant component. The extracted essential oil by MAHD was qualitatively similar to that obtained by HD however; it was achieved in a much short time. Therefore pilot microwave reactor might be proven suitable for large scale extraction with potential industrial applications.

1. Introduction

Natural products have been served as a major source of drugs; the latter contribute to human health. Essential oils and plant extracts are the most promising groups of natural compounds for the development of safer new drugs. Many efforts have been scientifically increased to discover new compounds from various kinds of plants for treatment of some diseases (Clark, 1996). One resource in the discovery of novel effective compounds may result from the traditional uses of natural compounds.

The genus *Ferulago* comprises of 35 species worldwide which seven species are found in Iran (Mozaffrian, 1996). *F. macrocarpa* belongs to the family Apiaceae (Mozaffarian, 2008). *F. macrocarpa* is a medicinal herb. A number of bioactive constituents have been reported from the essential oil of *F. macrocarpa*, with anti-anopheles and anti-malaria

mosquito activities (Hadji-akhoondi *et al*., 1992). The essential oil from *F. macrocarpa* contains borneol (Fig. 1a) and bornyl acetate (Fig. 2b). These components have sedative effects, especially on the nervous system (Buchbauer *et al*., 1992). A research on bovine brain neurotransmitters indicated that the anesthesia effect of borneol is more than a standard drug such as Lidocaine. In addition, it makes preventing stroke in mice (Xiao-Jing *et al*., 2006) and the life of rats suffered from stroke has increased significantly (Meirong *et al*., 1993; Chun-Jie *et al*., 2006). Results of several studies have also recommended the use of borneol in the eye drops, because borneol selectively increases the penetration of eye drop into the cornea (Li *et al*., 2006; Chun-Jie *et al*., 2006). Modern sample preparation techniques have been described for the extraction of components from the medicinal plants (Hao *et al*., 2002); these include ultrasound extraction, super-hot water extraction and microwave-assisted extraction process (Kaufmann *et al*., 2002). In recent years, the microwave-assisted hydro-distillation (Mohamad *et al*., 2006), solvent-free microwave extraction (Wang *et al*., 2006; Lucchesi *et al*., 2004, 2007), and a combination of microwave heating and dry distillation are developed as new techniques for the production of the essential oils. Development and application of modern sample-preparation techniques for extraction process toward gave a much cleaner analytical extract profile was increased.

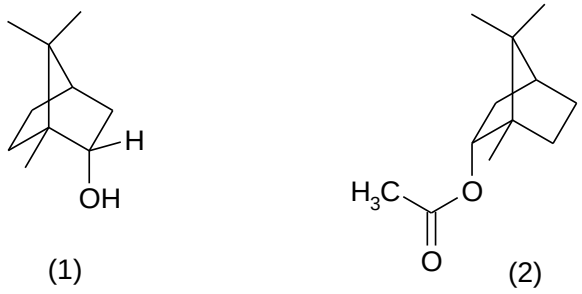

Fig 1. Structure of borneol andbornyl acetate

Extraction of essential oils through microwaves achieved by the following mechanisms, with irradiation of the plant surfaces, the essential oils are heated and ripple to the plant cells and then it get dissolved in the solvent (Ganzler *et al*., 1990), and microwave irradiation can decay the cell walls from the oil containing glands and with their disintegration, oil is dissolved in the solvent (Kaufmann *et al*., 2002).

In the present research, much effort has been focused on the isolation of potentially useful products from the plant by using MAE method without solvent. The samples, getting soaked in water, were irradiated with microwave in a Clevenger extraction approach under optimum conditions. The extracted components of the essential oils from this method were compared with those obtained by the conventional solvent extraction method.

2. Materials and Methods

2.1. Plant material

The aerial portions of *F. macrocarpa* were collected from Sanandaj in Kurdistan province, western Iran on April 2009. Voucher herbarium specimen (No. 4097) of the plant was identified and deposited at the Agricultural Research Center of Sanandaj, Iran. The aerial parts of plant were dried at room temperature for seven days.

2.2. Essential oil isolation

2.2.1. Extraction of essential oil by MAHD. The dried flower of *F. macrocarpa* were powdered using a blender into powdered pieces, whereby 50 g of dried powdered sample getting soaked in 750 mL water in the round-bottomed flask for 1h. The filtrated samples put in the microwave extractor were equipped with the Clevenger type apparatus, temperature and time controller. The essential oil was extracted from the collected material at 400, 500, and 600 W irradiation powers for 34 min. The essential oil was collected in a flask which was then dried over anhydrous sodium sulfate and evaporated the solvent using a vacuum rotary evaporator.

2.2.2. Extraction of essential oil by HD. In the traditional heating method, 50 g of the aerial part of *F. macrocarpa* was subjected to hydro-distillation for 3:30 h using a Clevenger type apparatus and the resulting oil was subsequently dried over anhydrous Na_2SO_4. The extracted oil from the two above described methods were kept in air-tight sealed glass vials and covered with aluminum foil at 4 °C for further analysis.

2.3. Essential oil analysis

2.3.1. Gas Chromatography (GC) analysis. The analysis of the essential oil was done by a GC (Agilent Technologies 6890 N) equipped with a FID detector, and HB-5 fused capillary silica columns (30 m x 0.25 mm, 0.25 µm film thickness). The samples, dissolved in ethanol were injected (20 µL**)** in the split less mode into Helium carrier gas with a constant flow rate of 2 mL/min. The injector and detector temperature was 280 °C. The column temperature was set at 40 °C for 3 min, and then programmed until 160 °C at a rate of 5 °C/min isothermal at this temperature for 2 min, and the finally increased at the rate of 10 ºC/min to 280 °C.

2.3.2. Preparation of standard and sample solution. Quantitative data of the compounds (borneol and bornyl acetate) were determined from the appropriate calibration curve (Fig. 1 and Fig. 2). Standard solution was prepared by dissolving of borneol and bornyl acetate (4 mmol) in 25 mL ethanol separately. Calibration standard solutions were prepared by the dilution of stock standard solution with ethanol in concentration range of 0.1 to 2.0 mmol/L for borneol and 0.25 to1.5 mmol/L for bornyl acetate. All standard solutions were filtered through 0.45 µm pore-size membrane filter before injection. The sample solution was prepared by transferring the sample with equivalent of 2.2 mg in 5 mL ethanol, and then a portion of it was filtered before injection into GC. The linear regression analysis of the data for the calibration plots of borneol and bornyl acetate showed linear relationship with r^2 = 0.9992 and 0.9959, respectively.

Figures 3 and 4 were shown the yields of extracted essential oil and the content of borneol and bornyl acetate in the oil by thermal heating and microwave-assisted methods. The yield of extracted oil by MW (0.04% w/w) compared to HD method (0.03 w/w) was increased. The content of borneol and bornyl acetate by traditional heating method is 3.1% and 42.82% of the total oil, respectively. MW effect in the content of borneol and bornyl acetate showed the yields of these compounds as7.86%, 57.72%, at 400w, 9.6% and 49% at 520w and 5.04% and 33.5% at 620w power irradiation, respectively.

3. Results and Discussion

Using MAHD and HD apparatus, the essential oils from *F. macrocarpa* was appeared as clear, light yellow colored and oily layer on the top of aqueous distillate which was trapped with 10 mL $CHCl_3$ as an organic collecting solvent. Using MAHD, the contents of essential oil obtained after 34 min were 20, 35, and 40µL with yields of 0.4, 0.7 and 0.8 µL/mg at 400, 500 and 600 W, respectively. Extraction by HD methods resulted in 30 µL essential oil with yield of 0.6 µL/mg, however it took a much longer time of 3:30 h.

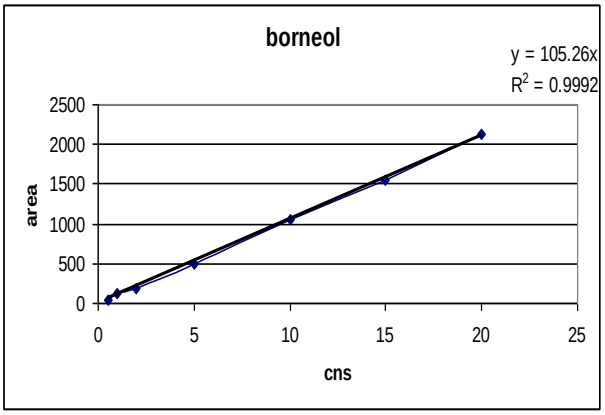

Fig 1. Calibration curve of standard solution of borneol at concentrations 0.1 to 2 mmol/L in ethanol

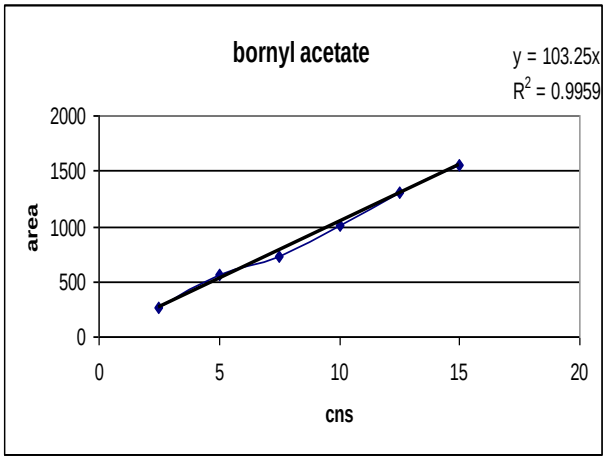

Fig 2. Calibration curve of standard solution of bornyl acetate concentrations 0.25 to1.5 mmol/L in Ethanol

The essential oil was subjected to detailed GC analysis in order to investigate borneol and bornyl acetate components. The signal at R_f = 15.2 and 17.1 min was identified borneol and bornyl acetate respectively with help of the chromatograms of their individual standard compounds (Fig. 6 and Fig. 7).

In order to evaluate the microwave power effect on the essential oil yields extraction was done under different power. As depicted in Fig. 3, with increasing the irradiation power from 400 to 620 W, the extraction of the essential oil increased and reached its maximum at 620 W. Although the essential oil yield increased to 0.04%, with increasing the irradiation power, from 400 to 620 W but, the applied high power irradiation decreased the yield of borneol and bornyl acetate (Fig. 4). This may be due to different materials having different appropriate microwave irradiation power and molecular interaction structure which can affect their yields of extraction. (Djouahri *et al*., 2013). Hence, 400 W was chosen as the appropriate microwave irradiation power. The extraction of borneol and bornyl acetate in MW method was enhanced by 2 to2.5 and 1.5 folds, respectively as compared to the thermal heating method. It is due to the better interaction of MW with polar compounds. Since the polarity of borneol is more than bornylacetat, therefore, the quantity of borneol in the essential oil increased.

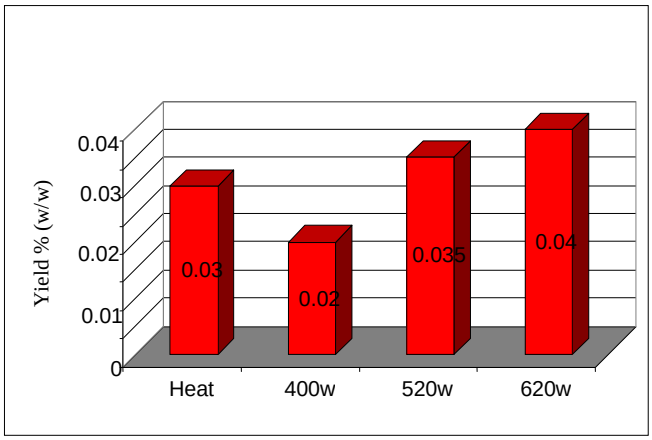

Fig 3. Compared the effect of microwave power and traditional heating method on the extraction of essential of *Ferulago macrocarpa*

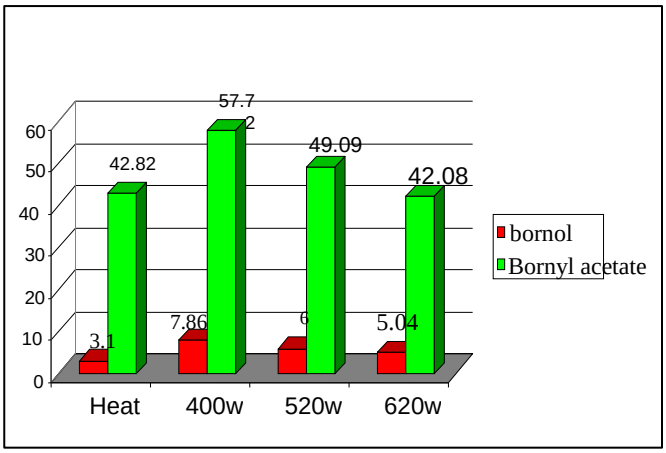

Fig 4. Compared the effect of microwave power with traditional heating method on the extraction yield of borneol and bornyl acetate

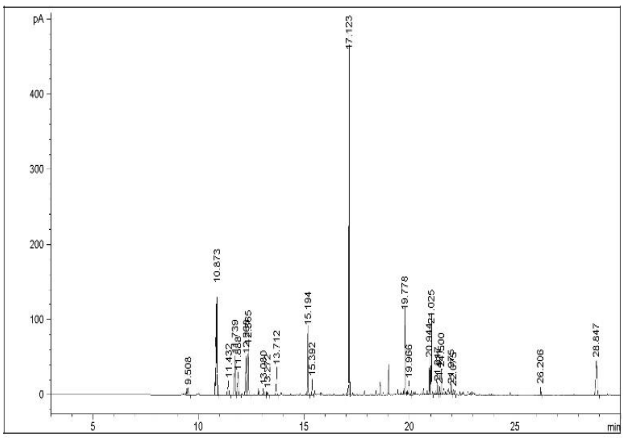

Fig 5. Chromatogram of the essential oil from *Ferulago macrocarpa* extracted by MAHD

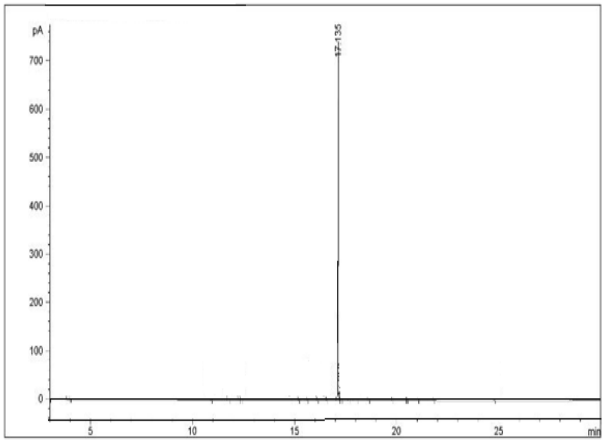

Fig 6. Chromatogram of standard solution of bornyl acetate

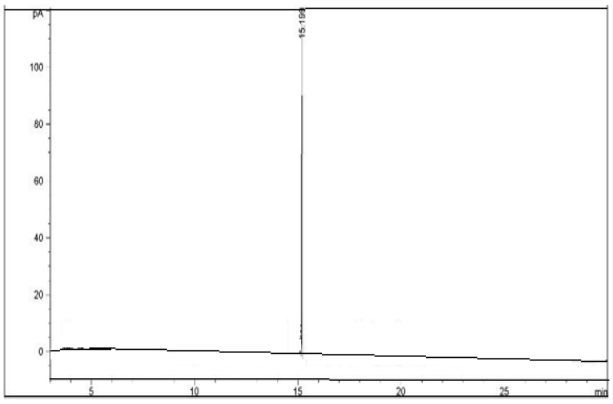

Fig 7. Chromatogram of standard solution of borneol

4. Conclusion

A simple and rapid microwave-assisted hydro-distillation (MAHD) protocol has been established for the isolation of borneol and bornyl acetate from *F. macrocarpa* at optimal condition. To evaluate the MAHD efficiency, we compared our results with those of hydro-distillation (HD) using the conventional Clevenger in terms of extraction time, extraction yield and efficiency. The yields of borneol and bornyl acetate were increased by 2 and 1.3 folds respectively, compared to HD.

5. Acknowledgements

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