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Statistical Regression Linear Based Models for *Origanum compactum* Essential Oil Yield and Major Compounds (Thymol and Carvacrol)

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Abstract

Here, we aimed at comparing *Origanum compactum*essential oil (OCEO) isolated using microwave assisted extraction (MW) and Clevenger hydrodistillation (HD). To this end, dried flowering tops from *O. compactum* were subjected OCEOs isolated separately using MW and HD and then analyzed using GC-MS. MW showed its superiority in terms of OCEO yield (7.41%), and total compounds (95.57%). In both techniques, thymol and carvacrol were the major compounds accounting for 78.81±0.22 and 14.84±0.39%, respectively, in the case of MW against 75.07±0.99 and 13.03±0.30% for HD. The correlations matrix showed strong positive correlations among OCEO yield and major compounds (thymol and carvacrol). Linear regression models were established for thymol and carvacrol against OCEO yield as well as thymol against carvacrol. In all cases, all models performed better since R^2 exceeded 75%. Based on these outcomes, OCEO was a thymol chemotype with important amounts of nutrients; MW could be suggested as a green efficient method over HD for OCEO isolation. Our regression models will allow the prediction of thymol and carvacrol based on OCEO yield as well as thymol from carvacrol content.

Keywords: *Origanum compactum,* regression models, essential oils, thymol chemotype.

Introduction

Morocco is one of the most important floristic areas in northern Africa thanks to its geographical position, diverse geology, topography, climate, and ecoregion (Ranko *et al*., 2013). The Moroccan flora is estimated to encompass 978 endemic taxa, which form more than half of North African endemic species (Bakha *et al*., 2017). This endemic richness seems to be a result of the presence of mixed and well-differentiated environments as highlighted in Ranko *et al.* (2013). *Origanum* is one of the main genera within the Lamiaceae family with important endemic taxa.

Besides, the prevalence of antibiotics effectiveness has been reported to decrease and multidrug resistance of microbes became a major concern to the global public health, which leads to a 'post-antibiotic' era (Reardon, 2014). In such a context, there is a pressing need to find novel strategies to fight drug-resistant microorganisms. To meet this need, natural products have received much attention to the search for new powerful antimicrobial agents, has become an important question (Bouyahya *et al*., 2020a; Jugreet *et al*., 2020; Lekmine *et al*., 2020). Indeed, recently, plants and their secondary metabolites have attracted the scientific community’s attention with an emphasis on their therapeutic potential (Saleem *et al*., 2019; Gharby *et al*., 2020; Llorent-

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Martinez *et al*., 2020). A huge number of plants used in folk medicine for curing different diseases, have been proven to be more efficient, less expensive when compared to conventional drugs, and show lesser or no side effects (Nisar *et al*., 2017). Also, various plant extracts, essential oils (EOs), and related compounds have been reported to have important antimicrobial powers (Zeroual *et al*., 2018; Al-Dhafri *et al*., 2020).

Origanum compactum Benth (*O. compactum*), locally known as "Zaatar" is one of the Moroccan endemic plant species belonging to the Lamiaceae family. It is a spontaneous annual plant (10-60 cm tall with bisexual, white/purple flowers grouped at the top of the stems of the flowers). *O. compactum* is essentially concentrated in Morocco and Andalusia (Spain). It is quite demanding in terms of moisture and grows mainly on slopes (Hamilton *et al*., 2003; Bouyahya *et al*., 2020a). *O. compacttum* (stem, flowers, and leaves) is widely used in folk medicine but also has many biotechnological applications, which arise from its phytochemical richness (Bouyahya *et al*., 2020a; Ait-Sidi-Brahim *et al*., 2019). EO of *O. compactum* (OCEO) can be isolated mainly from the flowering tops. OCEO is highly appreciated with many applications thanks to its numerous biological activities, which are associated with carvacrol, thymol, p-cymene, and γ-terpinene as the main constituents as compiled in Bouyahya *et al*. (2020). OCEO is mainly isolated through hydrodistillation, yields in published literature were found to be in the range of 0.31–5.7% (Bakhy *et al*., 2014; Aboukhalid *et al*., 2016; Bouyahya *et al*., 2017; Laghmouchi *et al*., 2018; Chahbi *et al*., 2020).

EO isolation technology has evolved to meet some considerations such as obtaining a higher yield, and achieving extraction in a shorter time, but also to provide valuable EOs. In this context, the microwave method has emerged as a green, cleaner method, and more efficient method (Lucchesi *et al*., 2004). This method was used to isolate EOs from some herbal species. Indeed, significant increases in terms of EOs yield and phytochemicals (especially oxygenated phytocompounds) as compared to conventional methods such as Clevenger hydrodistillation were evaluated for several species (Filly *et al*., 2004; Fathi Achachlouei *et al*., 2019; Hayat *et al*., 2020; Karrar *et al*., 2020).

To the best of our knowledge, no detailed information regarding chemical profiling of EO isolated from *O. compactum* growing in central-northern Morocco using microwave assisted extraction. Also, nutrients composition from this species has not been investigated before, hence the originality of this research work, which had objectives, (i) to compare phytochemicals profiling of EOs from *O. compactum* using both microwave and Clevenger hydrodistillation extractions and (ii) to set up regression models for the prediction of major compounds from essential oil yield.

Materials and Methods

Plant material and samples preparation

The plant species has been firstly authenticated. At a full blooming stage, the aerial parts (flowering tops) of *O. compactum* were collected in May 2019 from the Bouadel region (at 25km from Taounate Province). This region belongs to Central-northern Morocco and is characterized by a Mediterranean climate (humid in winter and semi-arid in summer). Collected plant samples were dried in a dark room to avoid photo-oxidationand then crushed to a fine powder using an electric grinder (Pizzale *et al*., 2002). The obtained powder was, therefore, subjected to phytochemical screening and essential oil (EO) isolation.

EOs isolation

O. compactum EOs (OCEOs) were isolated via two different methods namely: Microwave assisted extraction (MW) and Clevenger hydrodistillation (HD) as described below. OCEOs yields were calculated and expressed in percent per weight of the dried plant material (% DW). The obtained EOs using both methods (MW and HD) were subjected to phytochemical gas chromatography-mass spectrometry (GC/MS).

OCEO isolation using MW

Solvent-free microwave extraction was carried out according to Lucchesi *et al*. (2004) in a Milestone "DryDist" microwave laboratory oven, which is a multimode microwave reactor of 2.45 GHz with a maximum power of 103 W. During extraction, the temperature was controlled via an external infrared sensor. A plant material sample of 100 g was heated at atmospheric pressure using a fixed power density of 1 W g^{-1} for 15 min without adding water or solvents. The direct interaction between microwaves and biological water (present in plant material) fosters the release of EOs contained in the plant tissues. The mixture of hot ''crude juice" and in situ water moves, due to earth gravity downwards, on a spiral condenser where it can be easily condensed. In a receiving flask, oily condensate was gathered permanently. Inthe end, the obtained EO was collected, and dried using anhy-drous sodium sulphate.

OCEO isolation using HD

To isolate EO from *O. compactum*, dried aerial parts were submitted to hydrodistillation using a Clevenger-type apparatus (Hamdouch *et al.*, 2022). Three independent distillations each involving 100 g of plant material were carried out by boiling, for three hours, in a 1-liter flask topped by a column of 60 cm length connected with a refri-gerant as described by Jennan *et al*. (2018). EO obtained was separated from water using decantation. EO was, then, dried over anhydrous sodium sulphate and kept in dark vials at 4°C until use.

Phytochemical profiling of OCEOs using gas chromatography-mass spectrometry (GC/MS)

The analysis of EOs, obtained by both extraction techniques (MW and HD), was carried out according to Talbaoui *et al*. (2016). It was made on a TRACE GC ULTRA equipped with non-polar VB5 (95% methyl polysiloxane, and 5% phenyl), a capillary column (30 m×0.25 mm i.d. and 0.25 µm as a film thickness), directly coupled to a mass spectrometer (Polaris Q) (EI 70 eV). The temperatures of the injector and detector were maintained at 250 and 300°C, respectively. The oven temperature was programmed to rise at 4°C/min from 40 until 180°C and at 20°C/min for 180–300°C. Helium was used as a gas carrier with a flow rate of 1 mL/min. The samples (1 µL each) were injected according to a splitless mode.

Statistical analyses

All determinations and experiments were carried out, at least, in triplicates. The combined analyses of variance (ANOVA) were computed to elucidate the variances of yield and EO chemical composition. Quantitative differences, among mean values, were assessed by a general linear procedure followed by Duncan's test. Results were expressed as means±standard deviations (SD). Differences were consi-dered significant at a probability level of 5%. Correlations matrix among major EO compounds and EO yield, as well as linear regression models, were computed on mean values using STATGRAPHICS package version XVIII (Statpoint Technologies, Inc., Virginia, USA).

Results and Discussion

Chemical composition of OCEOs

Results regarding yields of EOs and chemical composition using both extra-ction techniques (microwave and Clevenger hydrodistillation) are illustrated in Table 1.

Table 1. Mean values of EO yield, % of total compounds, and individual chemical compounds (determined using GC/MS) of OCEO isolated using two extraction techniques microwave (MW) and Clevenger hydrodistillation (HD) from aerial parts of *O. compactum* collected from central-northern Morocco. Values are given as mean±SD of triplicate determinations. Compounds are listed in the elution order. For each parameter, values followed by the same letter are not significantly different at 5% as a probability level. RT=retention time and EO=essential oil.

EO traits	RT	MW	HD
Chemical compounds			
p-cymene	5.157	0.50±0.03 a	0.59±0.08 a
Thymol	5.438	78.81± 0.22 a	75.07± 0.99 b
Carvacrol	6.471	14.84± 0.39 a	13.03± 0.30 b
α-thujene	6.762	0.00±0.00 b	0.74±0.13 a
α-pinene	7.093	0.06±0.02 a	0.06±0.03 a
Caryophyllene oxide	7.224	0.08±0.03 a	0.03±0.01 b
Methyl lonolenate	7.845	0.38±0.04 a	0.35±0.07 a
Ethyl linolenate	10.243	0.83±0.09 a	0.40±0.09 b
Terpinolene	12.249	0.04±0.03 a	0.04±0.02 a
β-linalool	12.299	0.03±0.02 b	0.13±0.05 a
Total	-	95.57±0.33 a	90.45±0.73 b
EO Yield	-	7.41±0.11 a	5.68±0.18 b

According to these results, significant variations were highlighted between the two techniques used for EO isolation in terms of yield, % of total compounds, and individual chemical compounds. Moreover, microwave extraction showed its superiority for almost chemical compounds, % total compounds, and EO yield. In contrast, Clevenger hydrodistillation (HD) had the best scores of p-cymene, β-linalool, and α-thujene, which wereabsent in the case of microwave extraction.

Following Lucchesi *et al*. (2004) and Bousbia *et al*. (2009), the MW method has several advantages over traditional alternatives such as shorter isolation time (15 min against 3 h required for hydrodistillation), environmental impact (lower energy cost), a cleaner method (since no residue generation and no solvents used), enhances antimicrobial and antioxidant activity, and provides more valuable EOs (higher amount of oxygenated phytocompounds).

MW extraction as a green analytical technique is widely used to isolate EO from aromatic and medicinal plants, but also to extract neutraceuticals from some foods (Filly et *al*., 2004, Fathi-Achachlouei *et al*., 2019, Hayat *et al*., 2020, Karrar *et al*., 2020).

In the literature, EOs yields and chemical composi-tion were compared between MW and HD. In this context, EOs isolated using MWwere found to have higher yields, % of total compounds, and oxygenated monoterpenes (like thymol and carvacrol) but lower values of monoterpene hydrocarbons (such as α-pinene, α-thujene, and terpino-lene) as compared to the conventional HD technique (Filly *et al*., 2014; Khazayi *et al*., 2019). As explained in Filly *et al*. (2014), the higher percent-tage of oxygenated mono-terpenes obtained in MW is likely since the technique causes less hydrolytic and intense thermal effects than HD, which uses a large amount of water. More-over, oxygenated com-pounds possess a high di-polar moment and interact more vigorously with mi-crowaves and can, there-fore, be extracted more easily than monoterpene hydrocarbons, which are known to have low dipolar moments.

OCEO yields, obtained by both techniques, were expressed as percent-tages of plant dry weight. As shown in Table 1, the EO yield obtained by microwave (7.41±0.11%) was higher than that achie-ved using hydrodistillation (5.68±0.18%). The % of total compounds was significantly higher in the case of microwave isolation (95.57±0.33%) than in Clevenger hydrodistillation (90.45±0.73%). For the microwave method, the obtained chromatogram for OCEO chemical composi-tion was characterized by 9 chemical compounds (Table 1) against 10 com-pounds for Clevenger hydrodistillation as revea-led by the chromatogram (data not shown) accounttting for 96.9% of the total chemical composition. In both techniques, thymol and carvacrol were the major compounds since their concentrations exceeded 1% and the remaining consti-tuents were in concentrations lower than 1%. Thymol and carvacrol were found to be 78.81±0.22 and 14.84±0.39%, respecti-vely, in the case of microwave against 75.07±0.99 and 13.03±0.30% for Cleven-ger hydrodistillation. From these out-comes, it seems that OCEO was a thymol chemotype. Owing to its numerous health-healing properties and biotechnological applications such as the food industry, *O. compactum* phytochemistry has received much attention. A literature review shows that OCEO yield and chemical composition vary widely depending on several factors such as plant parts used for EO isolation, phenological stage, the geographical area under which plants are grown, harvest season, extraction techniques, and conditions like temperature, duration, among others (Belkamel *et al*., 2013; Bakhy *et al*., 2014; Aboukhalid *et al*., 2016; Laghmouchi *et al*., 2018; Bouyahya *et al*., 2017a; Bouyahya *et al*., 2020b; Stankov *et al*., 2020; Zeroual *et al*., 2020). The EOs yield values reported in our results were consistent with Bouyahya *et al*. (2017). These authors investigated OCEO yields and chemical composition according to various phenological stages, they found that the best record of yield is 5.7% (at the vegetative stage), which decreased to reach its minimum (2.9%) at the post-flowering stage. While studying 36 samples from various sites in northern Morocco, Bakhy *et al*. (2014) reported slightly lower values of OCEO yields (0.31–2.44%). Likewise, similar trends (1.22–4.24%) were observed by Laghmouchi *et al*. (2018).

With respect to chemical composition, a wide range of constituents of different groups (mainly oxygenated monoterpenes and monoterpenes hydrocarbons) were reported in OCEOs from different areas as reviewed recently in Bouyahya *et al*. (2020). Aboukhalid *et al*. (2016) studied the chemical composition of 88 *O. compactum* populations from several bioclimatic regions across Morocco, much higher chemotypic diversity was outlined with the dominance of six compounds: carvacrol (0–96.3%), thymol (0–80.7%), p-cymene (0.2–58.6%), γ-terpinene (0–35.2%), carvacryl methyl oxide (0–36.2%), and α-terpineol (0–25.8%). Despitethis chemical diversity, an overview ofthe published literature let conclude that the major compounds found in OCEO are the following: Carvacrol, thymol, p-cymene, and γ-terpinene (Bouyahya *et al*., 2017b, 2020). This chemical diversity is behind numerous biological activities of OCEO including antioxidant, antimicrobial, anticancer, and antiparasitic activities (Bouyahya et *al*., 2020b).

As discussed in the review compiled by Costa *et al*. (2019), both thymol and carvacrol are endowed with an important antioxidant power along with wound healing and anti-inflammatory properties, which justify their wide uses in the pharmaceutical industry. According to the same authors, these two monoterpenes are able to modulate the release of reactive species such as nitric oxide, pro-inflammatory cytokines, and growth factors associated with the initial stages of the healing process. Likewise, EO rich in thymol and carvacrol were demonstrated to possess antimicrobial activities against several pathogens (Bouyahya *et al*., 2020b; Jugreet *et al*., 2020). Mechanisms of action of thymol and carvacrol, as antimicrobial agents, are not yet fully elucidated, however, the main cascade of events uunderlying such mechanisms are the following: (1) structural and functional alterations affect cellular membranes; (2) the interference in both functionnality and synthesis of nucleic acids; (3) the coagulation of cytoplasm and leakage of some vital cytoplasmic constituents; (4) the imbalance of metabolism; (5) the interruption of the cellular communication through the inhibition of quorum sensing (Marinelli *et al.*, 2018; Bouyahya *et al.*, 2020b).

Correlations and Linear Regressions Models

The correlations matrix among OCEO yield and major compounds is summarized in Table 2. As it can be seen in these outcomes, OCEO yield was positively correlated to thymol (r=0.9465**) and carvacrol (r=0.9585**). Likewise, a strong positive correlation was found between thymol and carvacrol (r=0.8696*). These correlations were modeled through simple regression models. The results of these models are illustrated in Figure 1. A-C.

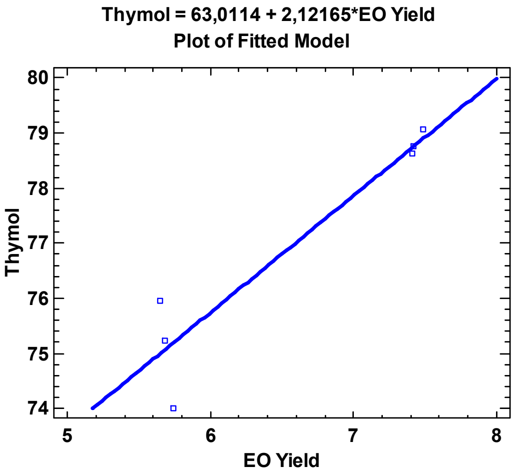

Figure 1A. Linear regression modelbetween EO yield and thymol. R^2=89.59%.

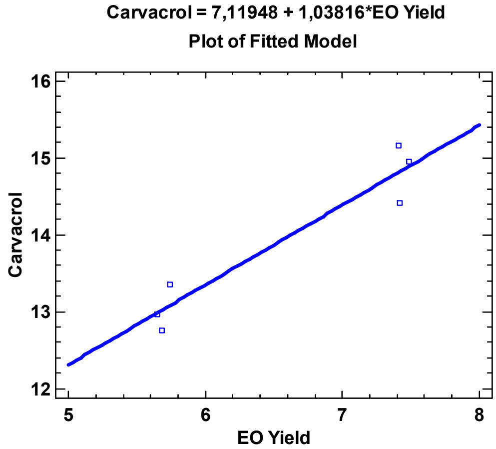

Figure 1B. Linear regression models among EO yield and carvacrol. R^2=91.86%.

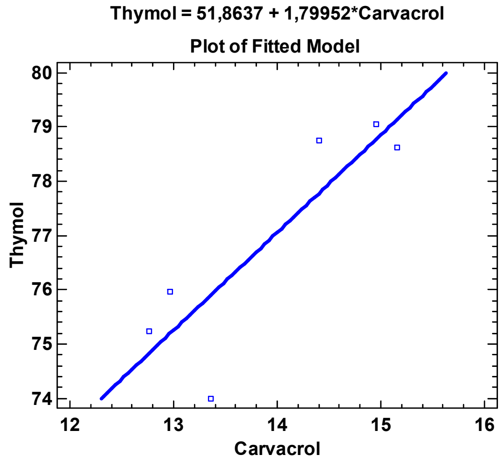

Figure 1C. Linear regression model between thymol and carvacrol. R^2 = 75.61%.

Table 2. Correlation coefficients among major OCEO compounds and yield.

	OCEO Yield	Thymol	Carvacrol
OCEO Yield	-	0.9465**	0.9585**
p-value		0.0042	0.0026
Thymol	0.9465**	-	0.8696*
p-value	0.0042		0.0244
Carvacrol	0.9585**	0.8696*	-
p-value	0.0026	0.0244	

Conclusions

From the results presented above, a set of conclusions could be drawn. OCEO isolated via microwave method showed its superiority over the conventional Clevenger hydrodistillation in terms of yield % of total compounds and almost individual compounds. In both cases (microwave and Clevenger hydrodistillation methods) OCEOs chemical composition was dominated by thymol (over 75%) and carvacrol (more than 13%) in both isolation techniques. Microwave technique might be suggested for EO isolation as a green, more efficient, and fast method. The established linear regression models will allow the prediction of thymol and carvacrol from OCEO yield with an accuracy exceeding 75%.

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Conflicts of Interest
The authors declare no conflict of interest.

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