ARTICLE IN PRESS

Industrial Crops & Products xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Essential oil composition of purple basils, their reverted green varieties (Ocimum basilicum) and their associated biological activity

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ARTICLE INFO

Keyword:
Basil
Oil composition
Bioactivity
Eugenol
Linalool
Methyl eugenol

ABSTRACT

Essential oils provide the characteristic aroma notes to basil varieties and are partially responsible for basil biological activities. The search for new aromas and biological activities are important steps to find new applications for industrial crop and products. The objective of this study was to evaluate the chemical composition of the essential oils from purple basil varieties and their progeny that reverted to green varieties, and to compare the composition of these oils with other nonpigmented basil varieties. This work also evaluated selected biological activities of some of these green and basil varieties in search of new biological activities. The green purple varieties derived from Dark Opal and Osmin Purple varieties exhibited a remarkable similarity, suggesting that these plants reverted to the same green ancestor. Their aromatic volatile oils and their main components exhibited a wide range of antioxidant and antimicrobial activities, with several chemotypes (Sweet, Osmin Purple basils) expressing high antioxidant and antimicrobial activities. Some of the reverted green types, showed higher levels phenylpropanoids (e.g. higher in eugenol) compared with their parent types (Purple Ruffles). These biological activities of the aromatic oils appear to be related to their phenolic constituents and concentration of the oils (e.g. eugenol, methyleugenol). The results showed that the essential oils of these basils have a number of additional attributes and applications for the nutraceutical industry in addition to their aroma and flavor impact.

1. Introduction

Purple basils (Ocimum basilicum, Lamiaceae) have been commercialized for both culinary and their ornamental value. The instability of purple basils, where a percentage of the progeny reverts to a green ancestor that same season of production, has limited their use as ornamental plants and also limited their potential applications for other markets. Phippen and Simon (2000) showed that Purple Ruffles basil varieties can have a reversion back to partially green sectoring on its leaves up to 35%. Basil essential oils are complex mixtures that can include over a hundred chemical constituents. However, only a few of these components are found in high relative concentrations, such as citral, 1,8-cineole, linalool, methyl chavicol, eugenol, methyl eugenol and methyl cinnamate and it is the blend or ratio of all the constituents that together provide the basis of the herbs aroma (Simon et al., 1990).

Oils from aromatic plants have been known since antiquity to possess biological activity, mainly antibacterial, antifungal and antioxidant properties (Koroch et al., 2007).

Previously, it was reported the antioxidant activity in leaves extracts of some *Ocimum* spp (Koroch et al., 2010; Juliani and Simon, 2002 Juliani and Simon, 2002).

The objective of this study is to evaluate the chemical composition of purple basil essential oils and their progeny reverted to green varieties, and to compare the composition of these oils with other basil varieties. This work also seeks to evaluate the biological activities of some of these green and basil varieties in search of new biological activities. The search for new uses is an important strategy to find industrial crop applications for herbs and aromatic plants. This an expanded study of our previous work (Juliani and Simon, 2002) now centered on the reversion of purple varieties.

2. Materials and methods

Seeds from purple *Ocimum basilicum* varieties: Dark Opal (DO), (Richters Seeds, Goodwood Ontario, Canada), Osmin Purple (OP), Purple Ruffles (PR) (Johnny's Selected Seeds) and the corresponding green reversions varieties: Green Dark Opal (GDO), Green Osmin Purple (GOP) and Green Purple Ruffles (GPR) were grown in flats before

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http://dx.doi.org/10.1016/j.indcrop.2017.04.066
Received 15 February 2017; Received in revised form 17 April 2017; Accepted 19 April 2017

Please cite this article as: Koroch, A.R., Industrial Crops & Products (2017), http://dx.doi.org/10.1016/j.indcrop.2017.04.066

transferring to six inches pot with a sterilized soil mix at the Rutgers University Research Greenhouse (New Jersey Agricultural Experimental Station, Rutgers University New Brunswick, NJ). For comparison purposes other basils were included in this study: *O. basilicum* varieties (Sweet Basil, SE, Red Rubin, RR, Cinnamon Basil,CI; Italian Large Leaf, ILL), *O. sanctum* (Holly Basil, HO) and *O. x citriodorum* (Sweet Dani Lemon Basil, LE) (Juliani and Simon, 2002). Plants were irrigated daily to soil saturation capacity and grown under day/night temperatures of 26–30 °C and 18–21 °C, respectively. The above-ground biomass of each individual plant was harvested at full bloom, placed in a paper bag and dried in a forced-air drier at 38 °C for 7 days prior to essential oil extraction and analysis.

The essential oils were extracted from dried leaves in a 1.5 L flask with distilled water (1:15 v/w) using a clevenger apparatus (Charles and Simon, 1990). Essential oil content was expressed as mL of oil per 100 g of dry weight. Volatile oils were analyzed by a gas chromatograph coupled to a mass spectrometer and flame ionization detector (Juliani et al., 2008). The ABTS radical cation assay was used to evaluate the free radical scavenging effect of the oils and their main components (Re et al., 1999). Stock solution of the oils and standards (eugenol, methyl eugenol, methyl chavicol, methyl-E-cinnamate, 1,8 cineole, linalool, citral) were diluted in ethanol. All assays were carried out at least three times and in duplicate. This method is based on reduction of the ABTS radical cation (2,2′-azino-bis (3-ethylbenzthiazoline − 6 − sulfonic acid) measured by absorbance at 734 nm. Results are expressed as percentage of Trolox equivalent antioxidant capacity/mL EO (TEAC, μmol Trolox/mL EO). For the ferric reducing power (FRAP) method, the oils and standards were diluted in a Tween 80 (10%) water solution and then vortexed for 3 min at full speed. The FRAP assay was used to assess the ascorbic acid equivalent antioxidant activity (AEAC, μmol Trolox/mL EO) (Benzie and Strain, 1996). For antimicrobial tests, the essential oils from selected *Ocimum* varieties and pure oil components (methyl eugenol, eugenol, methyl chavicol, methyl cinnamate, linalool and 2-allylphenol) were dissolved into 300 microliters (μl) of DMSO (Dimethylsulfoxide). Growth of microorganisms (Escherichia coli, Staphylococcus aureus and Saccharomyces cerevisiae) and the antimicrobial screenings were performed as described by Poulev et al. (2003). A dendrogram was constructed using the unweighted pair- group method with arithmetical average (UPGMA). The data were transformed with the stand procedure from NTSYS-pc (Rohlf, 1990; Juliani et al., 2008). The experimental design was fully randomized. Data were analyzed statistically by analysis of variance (ANOVA) followed by the LSD test (5%).

3. Results and discussion

The chemical composition of the different types of basils showed a remarkable diversity (Juliani and Simon, 2002) (Table 1). The comparison of the oil profile of the purple and their green counterparts showed some similarities (Table 1). The purple ruffles (PR-GPR) pair belonged to a methylchavicol chemotype, with GPR showing higher amounts of phenylpropanoids, methylchavicol 50.3% and much higher levels of eugenol (8%) as compared with the PR, 46.4% and 0.5% for methychavicol and eugenol, respectively.

The Dark Opal pair (DO-GDO) belonged to a linalool-eugenol chemotype showing a similar profile with linalool (54.3 and 57.2% for DO and DGO respectively), eugenol (6.9 and 7.3%), 1,8 cineole (8.7 and 9.4%) exhibiting similar amounts (Table 1). The Osmin Purple pair (OP, GOP) also a linalool-eugenol type showed the highest amounts of linalool for the GOP (59%), and lower amounts of eugenol (7.0%), while the OP showed lower levels of linalool (51.1%) and the highest amounts of eugenol (14.7%) among the purple basils (Table 1).

A cluster analysis conducted with these pairs and our previous data (Juliani and Simon, 2002) (Fig. 1), showed the presence of three clusters, that separated the three species of Ocimum, Lemon basil (LE) (a hybrid, *O. x citriodorum*) and Holy basil (*O. tenifolium*). All the varieties of *O. basilicum* were grouped and separated in two groups, according to their main components and 1,8-cineole content. The basils, Sweet basil (SE) and Cinnamon basil (CI) were low in 1,8-cineole content in contrast to all others which were higher (5–9%). This latest group could then be further sub-grouped based on the high methyl chavicol and low linalool content (Italian Large Leaf (ILL), Purple Ruffles (PR) and Green Purple Ruffles (GPR); absence of methyl chavicol and high linalool Red Rubin (RR)); or low linalool and eugenol content −absent in the former groups: Dark Opal (DO), Green Dark Opal (GDO), Osmin Purple (OP) and Green Osmin Purple (GOP) (Fig. 1, Table 1). In a similar study, Carović-Stanko et al. (2011) used cluster analysis (dendrogram), discriminated *Ocimum* species and *O. basiculum* varieties in two main clusters rich in methylchavicol and linalool. Interesting to note was that DO and GDO were grouped in different subclusters, suggesting that GDO reverted to a green ancestor similar to the OP/GOP type. PR and GPR were grouped in the same cluster as the ILL basils, suggesting a similar origin (Fig. 1). The total essential oil accumulation was significantly higher in GOP and GDO than in their purple counterparts (Table 2).

These chemical variations and the presence of phenylpropanoids have important effect on their biological activities. The highest radical scavenging activity was associated with the linalool-eugenol chemotypes, with the highest activity observed in those with highest content of eugenol, OP essential oils (Tables 1 and 2). Those varieties that belong to the linalool-methyl chavicol (PR and GPR) exhibited the lowest activity (50–59 μmol/mL EO, TEAC). The reducing ability of oils, measured as AEAC showed a similar trend to TEAC, with SE (Sweet Basil) and OP essential oils exhibiting the highest activity (2281 and 1263 μmol/mL EO respectively). The DO, GDO, and GOP basils showed significantly lower activity (from 269 to 469 μmol/mL EO respectively). Comparatively, PR and GPR exhibited the lowest antioxidant activity (31 to127 μmol/mL EO). The antioxidant activity of the essential oils of basil was similar using both *in vitro* chemical models. As the ability of the antioxidants to scavenge free radicals (ABTS) and the ability to reduce iron (FRAP) is closely related ($R^2 = 0.68$), these results provide additional confirmation of the differential response based on the major chemical constituents in the essential oils. The antioxidant activity of the major oil constituents showed that eugenol exhibited the highest antioxidant activity in both models (Table 2). Furthermore, there was a close relationship between antioxidant activity in both assays and the amount of eugenol in the different essential oils (TEAC $R^2 = 0.75$; AEAC $R^2 = 0.75$). Essential oils rich in 1,8-cineole, linalool showed a very low antioxidant activity. These results illustrate the importance of the hydroxyl group in the aromatic ring to confer the antioxidant activity to a molecule like eugenol, in contrast methyl-E-cinnamate (CI), which lacks this group showed a very low antioxidant activity (Table 2). In the case of methyl eugenol (HO oil), where the hydroxyl group is blocked by methylation, the antioxidant activity decreased considerably. Methyl eugenol antioxidant activity in both assays was higher than that of methyl chavicol, suggesting that an additional methoxy group increases the antioxidant activity in methyl eugenol. The higher antioxidant activity of eugenol compared with Trolox could be associated with the presence of the methoxy group in the eugenol molecule. Volatile constituents, lacking a phenolic ring such as linalool, citral (neral and geranial) and 1,8-cineol or having the hydroxyl group blocked by methylation (methyl chavicol), exhibited the lowest antioxidant activity (Table 2).

From a nutraceutical perspective, eugenol is an interesting component of essential oils due to its high antioxidant and antibacterial activities. The challenge in its incorporation in botanical products is due to the strong taste and odor impact that a small concentration of eugenol can impart to final products. The highest antioxidant activity (1105 TEAC-2281 AEAC) within this range of chemotypes was associated with the linalool-eugenol chemotype. The essential oils from selected basils showed different antimicrobial activities. All the oils tested inhibited 100% of the growth of yeast. SE oil exhibited the