PharmacologyOnLine

Archives • 2021 • vol.2 • 426-433

CHEMICAL COMPOSITION OF BAY LAUREL AND ROSEMARY ESSENTIAL OILS FROM MOROCCO AND THEIR ANTIFUNGAL ACTIVITY AGAINST FUSARIUM STRAINS

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Abstract

Contamination of agro-food items with mycotoxins produced by various classes of phytopathogenic fungi is a long-standing issue that has piqued interest in recent decades. Mycotoxins can have both acute and chronic toxic effects, and they can be responsible for food poisoning episodes in both livestock and humans. Natural chemicals have attracted interest in recent years, and concerns about the safety of synthetic compounds have prompted further in-depth research into plant resources. Essential oils, odorous and volatile products of plant secondary metabolism, have proven to have great antimicrobial properties. The most common pathogens used to test their antimicrobial activity are human and food-borne infections. This study was designed to examine the in vitro antifungal activities of *Laurus nobilis L.* and *Rosmarinus officinalis L.* essential oils compared their main component (1, 8-cineole). The antifungal activities were determined with agar well diffusion method on five fusarium strains (*Fusarium oxysporum, Fusarium avenaceum, Fusarium graminearum, Fusarium subglutinans, Fusarium verticillioides*). GC–MS analysis of the bay laurel essential oil resulted in the identification of 43 compounds, representing 89, 87 % of the oil, and that of the rosemary essential oil led to the identification of 35 components representing 85, 31% of the oil. The major component of both the essential oils was found to be 1, 8-cineole with a concentration of 30, 41% in the bay laurel oil, and 34, 25 % in the rosemary oil. Both the essential oils and the 1,8-cineole were inhibitory toward the studied fungal species but the antifungal activity offered by 1, 8-cineole was incomplete, which could indicate the major oil constituent is not the only component responsible for limiting fungal growth.

Keywords: *Laurus nobilis L., Rosmarinus officinalis L., Essential oil, GC–MS analysis, antifungal activity, Fusarium.*

Introduction

Essential oils are complex natural combinations of volatile secondary metabolites that are extracted from plants using hydro or steam distillation. The fragrance and biological capabilities of aromatic and therapeutic plants are due to the major ingredients of essential oils, mono- and sesquiterpenes, including carbohydrates, alcohols, ethers, aldehydes, and ketones. Spices and herbs have been used in food as preservatives and flavoring agents since ancient times because of these qualities. Essential oils have been extracted from various parts of plants for ages and are used for similar purposes. Essential oils cover a broad spectrum of activities. Pharmacological effects of essential oils include anti-inflammatory, antioxidant, and anticancerogenic activities. Others are biocides against bacteria, fungi, viruses, protozoa, insects, and plants [1].

The mechanism of essential oil impact on microorganisms is complicated and not entirely understood. Essential oils' antimicrobial activity is thought to be influenced by their hydrophilic or lipophilic nature. Terpenoids, for example, are lipid soluble compounds that influence the activity of membrane catalyzed enzymes, such as those in respiratory pathways. Certain essential oil components can function as uncouplers, interfering with proton translocation across a membrane vesicle and, as a result, interrupting ADP phosphorylation (primary energy metabolism). Specific terpenoids having functional groups, such as phenolic alcohols or aldehydes, also inhibit the synthesis or activity of membrane-integrated or related enzyme proteins [2].

Antibacterial and antifungal activity of essential oils obtained from plants is frequently tested. So far, all of the essential oils examined have shown some antibacterial action. This action differs from one essential oil to another, as well as from one examined microbial strain to another, but it is always dose-dependent [1]. In the present study we chose bay laurel and rosemary essential oils. *Laurus nobilis L.* (Lauraceae), or bay laurel is an evergreen shrub indigenous to the south parts of Europe and the Mediterranean area [3]. *Rosmarinus officinalis L.* (Rosemary) is an aromatic plant belonging to Lamiaceae family used for culinary and medicinal proposes, due to its aromatic properties and health benefits [4]. Wild rosemary is abundant in Morocco's Rif, Middle, and High Atlas mountains. It's been utilized in pharmaceuticals as well as traditional medicine [5] as anti-cancer [6], antifungal [7] and as insecticide [8].

We chose *Fusarium oxysporum, Fusarium avenaceum, Fusarium graminearum, Fusarium subglutinans* and *Fusarium verticillioides* to investigate the antifungal activity. Fusarium is a genus of filamentous fungal organisms that are widely spread in nature. They cause a wide range of illnesses in humans, animals, and plants, and are thus important for public health and the economy [9]. The mycotoxigenic groupe of fungi were chosen because they are relevant to Mediterranean crops, as well as the need to investigate innovative ways for controlling and reducing mycotoxins in the agro-food chain.

Because of the varied composition of essential oils major ingredients, standardization and comparability of results after the application of essential oils as antifungal agents is challenging. For this reason, in our work, in addition to the two essential oils, we focused on the use of one pure oil component, 1, 8-cineole, as potential fungicide agent against mycotoxigenic plant pathogenic fungi. The choice was made for two reasons: this molecule is frequently utilized as a therapeutic antibacterial agent and disinfectant [10-11] and it is the major component of our two studied essential oils.

Methods

Plant material

The aerial parts of *Laurus nobilis L.* and *Rosmarinus officinalis L.* were collected in October of 2020 at the region of Beni Mellal-Khénifra, center of Morocco.

Extraction of the essential oils

The aerial parts of *Laurus nobilis L.* and *Rosmarinus officinalis L.*, were oven dried at 60°C and the Hydrodistillation was performed using a Clevenger-type equipment. The oil was then separated by decantation and weighed. After that the yield of the essential oils (%) is determined by

the weight of the oil extracted divided by the weight of the plant material used. Finally the resulting essential oil was stored at -4°C in dark until use.

Analysis and chemical compound identification

Essential oils antibacterial action is directly proportional to their chemical composition. [1] So, for identification of components gas chromatography coupled with mass spectrometry (GC–MS) was used. The gas chromatography analysis was performed on SHIMADZU GC-14B supplied with an FID detector and a LM-5 (30 m ×0.25 mm ×0.3 mm) capillary column. By comparing the mass spectra acquired by CG–MS with literature data, the components in the oil were identified.

Antifungal activity

The antifungal activity of *Laurus nobilis L.* and *Rosmarinus officinalis L.* essential oils was investigated against *Fusarium oxysporum, Fusarium avenaceum, Fusarium graminearum, Fusarium subglutinans* and *Fusarium verticillioides.* The fungal strains were isolated in the laboratory of the higher school of technology Khenifra, Sultan Moulay Slimane University, Khenifra, Morocco. We also tested the fungal activity of the 1, 8-cineole on the same strains to use the results as references, since it was the major compound in both of the tested essential oils. The 1, 8-cineole compound was commercially purchased.

The experiment was carried out on a Sabouraud agar medium. The agar well technique utilized was based on Deans and Ritchie's well-known method (Seirafinia et al., 2017) [12] with slight modifications. The freshly prepared inoculums of the *F. oxysporum, F. avenaceum, F. graminearum, F. subglutinans* and *F. verticillioides* were swabbed all over the surface of the nutrient agar plate using sterile cotton swab. The wells were bored into the medium using a sterile cork-borer with a 6-mm diameter and correctly labeled. Then 6 µL of each essential oil and the 1, 8-cineole compound were added separately to the wells. All petri dishes were sealed with sterile parafilm to prevent the essential oils from evaporating. After adding the oils to the wells, the plates were left undisturbed for 30 minutes to allow the oils to diffuse into the agar. Then they were incubated at 25 °C for ten days. Plates were examined for zones of inhibition after incubation. The inhibitory properties were evaluated in triplicates.

Results and discussion

Extraction of the essential oils

The hydrodistillation of the aerial parts of *Laurus nobilis L.* and *Rosmarinus officinalis L.* provided two essential oils characterized with typical odors, and yields of 0.28% and 0.36% respectively.

Analysis and chemical compound identification

The essential oils of the two studied plants (*Laurus nobilis L.* and *Rosmarinus officinalis L.*) components, their retention indices (RI) and the percentage composition are presented are represented in the tables 1 and 2.

We were able to identify 89.87 % of the bay laurel essential oil compounds, it was found to contain 1,8-cineole (30.41%), α-Terpinyl acetate(12.61%), sabinene (6.56%), α-Pinene (6.53%) and β-Linalool (3.63%) as major constituents (Table 1). Previous studies have also proven that the major compound of this oil is 1, 8-cineole [13-15].

Chemical analysis of the components of the rosemary essential oil led to identification of 35 components, of which 85.31% were identified (Table 2). The major components of the oil were 1, 8-cineole (34.25 %), borneol (11.33%), linalool (5.74%), β-pinene (3.75%), α-Pinene (2.24%) and p-Cymene (2.87%). The essential oil constituents of *Rosmarinus officinalis L* were determined previous studies, with 1, 8-cineole being the most dominant constituent [16-18].

Antifungal activity

Results of the antifungal activity test can be seen in table 3 and figure 1. The essential oils and the 1,8-cineole were clearly inhibitory toward the studied fungal species, *Fusarium oxysporum, Fusarium avenaceum, Fusarium graminearum, Fusarium subglutinans* and *Fusarium verticillioides*.

Bay laurel essential oil appeared effective against all the fungal strains, and had a maximal inhibition zone of 2.02 mm against *F. graminearum*, and a minimal activity on *F. verticillioides* with an inhibition

zone of only 0.87 mm. The activity of rosemary oil was determined to be maximal on the *F. subglutinans* with an inhibition zone of 2.85 mm, whereas it had the minimal activity on the *F. avenaceum* with an inhibition zone of 0.45 mm. The major compound 1, 8-cineole tested alone, showed maximal inhibitory activity against *F. subglutinans* with an inhibition zone of 2.15 mm, but almost all the samples were less effective than both the essential oils

In general, we may say that 1,8-cineole's antifungal activity was incomplete, implying that the primary oil constituent isn't the only one responsible for inhibiting fungal development. Because essential oils comprise a variety of diverse chemical substances, it's difficult to link the antifungal activity of a complete essential oil to one or a few active compounds. Minor compounds, in addition to major compounds, may play a substantial role in the oil's activity.

Conclusion

In recent years, considerable and intensive research into the analysis of content and biological activity of essential oils has been conducted for one primary reason: human health. Chemical medications and preservatives are regarded to be the source of many carcinogenic and teratogenic qualities, as well as residual toxicity. Consumers are wary of chemical additives, prompting a surge in demand for natural, more socially acceptable agents to protect humans, cattle, and food against disease, pests, and spoilage. The multiple studies on essential oils have clearly demonstrated that they present a great potential for medical procedures and for food, cosmetic and pharmaceutical industries. In conclusion, we have demonstrated that the bay laurel and rosemary essential oils plus the commercial 1, 8-cineole are active, in the in vitro control of *Fusarium oxysporum, Fusarium avenaceum, Fusarium graminearum, Fusarium subglutinans* and *Fusarium verticillioides*.

Even though the antifungal activity of the1, 8-cineole compound was proven to be incomplete, the fact that this compound is commercially available at low cost support it's possible use as antifungal agent. Because essential oils are composed of a variety of chemical compounds, it's difficult to attribute an essential oil's antifungal action to just one or a few active principles. In addition to large components, minor compounds may play a significant part in the oil's activity [19-20].

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Table 1. Chemical composition of the bay laurel (*Laurus nobilis L.*) essential oil.

Peak	Compounds	RI	Percentage composition (%)
1	α-Thujene	931	0.22
2	α-Pinene	939	6.53
3	Camphene	954	1.61
4	Sabinene	971	6.56
5	*β*-Pinene	979	2.05
6	*β*-Myrcene	991	0.28
7	α-Phellandrene	1003	2.19
8	α-Terpinene	1014	0.54
9	*p*-Cymene	1020	0.38
10	Limonene	1029	1.32
11	1.8-cineole	1032	30.41
12	*cis*-*β*-ocimene	1046	0.05
13	*trans*-*β*-ocimene	1050	0.24
14	γ-Terpinene	1055	0.93
15	*cis*-Sabinene hydrate	1065	0.21
16	*β*-Linalool	1096	3.63
17	Terpinene-4-ol	1179	2.15
18	α-Terpineol	1189	2.13
19	Bornyl acetate	1286	1.85
20	α-Terpinyl acetate	1333	12.61
21	Thymol	1336	0.35
22	Eugenol	1363	0.67
23	*β*-Elemene	1390	2.83
24	Methyleugenol	1402	4.74
25	*β*-Caryophyllene	1429	0.39
26	Germacrene D	1484	0.08
27	Bicyclogermacrene	1501	0.05
28	Caryophyllene oxide	1574	0.45
29	Ledol	1602	0.32
30	(−)-Spathulenol	1619	0.26
31	τ-Cadinol	1628	0.46
32	*β*-Eudesmol	1642	0.39
34	Cedren-13-ol acetate<8->	1788	0.32
34	*n*-Heneicosane	2100	0.19
35	Phytol	2105	0.22
36	*n*-Docosane	2200	0.21
37	*n*-Tricosane	2300	0.19
38	*n*-Tetracosane	2400	0.17
39	*n*-Pentacosane	2500	0.25
40	*n*-Hexacosane	2600	0.41
41	*n*-Heptacosane	2700	0.34
42	*n*-Octacosane	2800	0.27
43	Squalene	2817	0.42
Total identified compounds (%)			89.87

Table 1. Chemical composition of rosemary (Rosmarinus officinalis L.) essential oil.

	Compounds	RI	Percentage composition (%)
1	α-Thujene	927	0.21
2	α-Pinene	929	2.24
3	Camphene	948	1.51
4	β-pinene	973	3.75
5	1-octen-3-ol	976	0.43
6	β-myrcene	988	1.84
7	α-phellandrene	1001	0.62
8	3-carene	1007	0.06
9	α-terpinene	1014	1.57
10	P-cymene	1020	2.87
11	1.8-cineole	1027	34.25
12	Cis-Ocimene	1035	0.54
13	β-Ocimene	1041	0.14
14	γ-terpinene	1055	3.54
15	cis-Sabinene hydrate	1061	0.78
16	Terpinolene	1084	1.32
17	Linalool	1089	5.74
18	Fenchol	1108	0.05
19	Campholaldehyde	1121	1.88
20	Camphor	1138	3.24
21	Isopulegol	1143	0.87
22	Pinocamphone/isopinocamphone	1160	1.21
23	Pinocarvone/trans-pinocarvone	1164	1.05
24	Borneol	1168	11.33
25	4-terpineol	1174	0.52
26	p-cymene-8-ol	1181	0.21
27	α-terpineol	1184	1.24
28	Myrtenol	1194	0.05
29	Verbenone	1198	1.01
30	Citronellol	1224	0.11
31	Bornyl acetate	1274	0.31
32	Carvacrol	1296	0.25
33	Methyl eugenol	1401	0.19
34	β-caryophyllene	1414	0.31
35	α-humulene	1448	0.07
Total identified compounds (%)			85.31

Table 3. Inhibition zone (mm) of the essential oils and 1.8-cineole compound on the studied fungal strains after ten days inoculation.

	EO of laurel	EO of Rosemary	1,8-cineole
F. oxysporum	1.87 ± 0.12	1.56 ± 0.11	1.54 ± 0.12
F. avenaceum	0.98 ± 0.11	0.45 ± 0.09	0.35± 0.08
F. graminearum	2.02 ± 0.15	1.32 ± 0.11	1.18 ± 0.10
F. subglutinans	1.87 ± 0.11	2.85 ± 0.18	2.15 ± 0.16
F. verticillioides	0.87 ± 0.11	1.22 ± 0.12	1.35 ± 0.12

Figure 1. Graphic presentation of the inhibition zone of the rosemary and bay laurel essential oils and 1.8-cineole on the studied fungal strains.