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Bioactive Compounds and Antifungal Activities of Extracts of Lamiaceae Species

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Abstract

Origanum vulgare L. (oregano) and *Rosmarinus officinalis* L. (rosemary) are vegetal species belonging to the family Lamiaceae, popularly known as oregano and rosemary. Aromatic plants are used in the treatment and prevention of diseases and in the culinary as functional food in the preparation and conservation of foods. In the chemical composition of oregano and rosemary are present bioactive compounds with antimicrobial, antioxidant and flavoring effect. Several reports in the literature have presented the chemical composition and biological activity of the essential oils of oregano and rosemary. However, few studies have been carried out regarding the chemical composition and biological potential of the aqueous and ethanolic extracts of *Origanum vulgare* L. and *Rosmarinus officinalis* L. Evidencing a need to investigate the chemical composition and antifungal activity of these extracts. The objective of the study was to evaluate the bioactive compounds and antifungal activity of the aqueous and ethanolic extract of *Origanum vulgare* L. and *Rosmarinus officinalis* L. The aqueous and ethanolic extracts of *Origanum vulgare* L. and *Rosmarinus officinalis* L. present in the chemical composition phenolic acids and flavonoids. The antifungal test of the aqueous and ethanolic extract of *Origanum vulgare* L. and *Rosmarinus officinalis* L. presented antifungal potential against *Candida globosa*, *Cryptococcus laurentii*, *Trichosporum assai*, *Rhodotorula* sp., *Candida albicans*, *Kodamaea ohmeri*, *Saccharomyces* and *Geotrichum*. According to the results obtained in this study, it was concluded that the ethanolic extract of oregano and rosemary present antifungal activity against several yeasts tested, thus proving that these plant species must be carefully evaluated, aiming at a potential for use as an antimicrobial agent.

Keywords

Origanum vulgare, *Rosmarinus officinalis*, Antifungal, Phenolics, Flavonoids

1. Introduction

Origanum vulgare L. and *Rosmarinus officinalis* L. are species of plants belonging to the family Lamiaceae (Labiatae) [1]. Plants originating in the Mediterranean region adapt well to various parts of the world [2]. Spices are popularly known as oregano and rosemary, with properties beneficial to human health, used in dish preparation and food preservative [3], also used in folk medicine in the prevention and treatment of various human and animal diseases. The biological properties of the plant species are related to the compounds produced by the secondary metabolism of the plant [4] [5] [6]. Several studies have been carried out analyzing and identifying the chemical composition of the essential oils of oregano and rosemary and their biological properties [6] [7]. The development of research on the application of plant species with pharmacological properties to alternative in the prevention and treatment of diseases as well as employment in the development of new drugs or products for the food industry has been increasing and instigating the researchers in the investigation of the chemical and biological properties of plant extracts little studied [8] [9] [10]. In this context, the growing interest in new knowledge has shown that numerous plants such as Lamiaceae have significant antimicrobial activity against different microorganisms [11]. In addition, microbial resistance to commercially available antimicrobials has been a worldwide concern and in this sense one of the alternatives that is emerging is the study of plant-derived antimicrobials [12] [13]. Several pathologies that affect Public Health are of microbial origin. Among the pathologies, it stands out the candidiasis, opportunistic infection that affects men and animals. This disease has been common in veterinary medicine as well as resistance to antifungal [14] [15] [16]. In animals it has been reported in the literature skin infections [17], ear [18], gastrointestinal [19], among others. Another opportunistic pathogen in immunocompromised and granulocytopenic patients is *Trichosporon asahii* [20]. The genus *Rhodotorula* has been considered as an emerging agent causing infections in immunosuppressed humans. These yeasts can be isolated from soil, stool, food and air samples [21] [22]. The fungemia by this pathogen is usually associated with endocarditis, meningitis, peritonitis, immunosuppression, prostheses and intensive use of catheters [23]. *Kodamaea ohmeri* is recognized as an opportunistic pathogen in immunocompromised patients [24]. Recent studies have demonstrated antifungal activity of *Origanum* sp. (Oregano), especially when used in the form of essential oil, few studies with other types of extracts [25] [26]. The objective of this work was to evaluate the antifungal activity of the aqueous and ethanolic extracts of *Origanum vulgare* L. and *Rosmarinus officinalis* L. against fungal species, such as *Candida globosa*, *Candida albicans*, *Trichosporon asahii*, *Kodamaea ohmeri*, *Saccharomyces*, *Cryptococcus laurentii*,

Rhodotorula sp. and *Geotrichum*.

2. Material and Methods

2.1. Obtaining of Samples

Samples of *Origanum vulgare* L. and *Rosmarinus officinalis* L. were purchased commercially with certificate of quality, origin and botanical identification.

2.2. Obtaining Extracts of *Origanum vulgare* L. and *Rosmarinus officinalis* L.

About 25 g of dried leaves of *Origanum vulgare* L. with 250 mL of distilled water were subjected to the oil bath, with a temperature between 65˚C - 70˚C, in a period of one hour, under stirring, to obtain the aqueous extract. After they were filtered, this procedure was repeated twice. The obtained extract was subjected to lyophilization, obtaining a powder. To obtain the ethanolic extract were used 35 g of oregano leaves and 350 mL of ethyl alcohol P.A, under agitation in oil bath, for a period of 12 hours, with temperature between 65˚C - 70˚C. After the rotary evaporator was used to concentrate the extract. The same procedure was performed with the dry leaves of rosemary to obtain the aqueous and ethanolic extract of *Rosmarinus officinalis* L.

2.3. Chemical Identification of Extracts

Bioactive compounds of *Origanum vulgare* L. and *Rosmarinus officinalis* L.was defined through high performance liquid chromatography (HPLC) by using Varian Diode Array Detector (DAD). The reverse phase column C-18 (Phenomenex Gemini, 25 cm × 4.6 mm × 5 μm) was used for the separation of bioactives compounds. The column was maintained at 40˚C and analyzed for the following wavelengths of interest: 280, 300, and 320 nm. The injection volume was injection volume of 10 μL and with flow rate of 1 $mL{\cdot}min^{-1}$. The mobile phases were water acidified with phosphoric acid and 1% methanol. Elution of the phenolic compounds was performed using the following gradient mode: 0% - 15% B 2 min; 15% - 25% B for 5 min; 25% - 30% B for 10 min; 30% - 35% B for 15 min; 35% - 50% B for 25 min; 50% - 60% B for 30 min; 60% - 80% B for 35 min; 80% - 100% B for 45 min; and 100% - 5% B for 60 min. The phenolic compounds were identified and quantified based on the analysis of patterns of phenolic compounds (Sigma-Aldrich, >98% purity) under identical analytical conditions used in the samples. The identification parameters applied were spectral similarity, matching the retention times and spectral purity of the peaks to the retention times of interest. The quantification was performed using external standards and 6-point dilution curves (done in triplicate) and an $R^2 > 0.9$ for each individual pattern.

2.4. Antimicrobial Activity

In order to test the antimicrobial susceptibility of the aqueous and ethanolic extract

of *Origanum vulgare* L. and *Rosmarinus officinalis* L., the broth microdilution method was used, according to M-27 (CLSI, 2002) and M-38 (CLSI, 2002). 96-well flat bottom microplates were used. From the stock solutions of oregano, ten successive dilutions were prepared in RPMI medium. 100 µl aliquots of these concentrations were dispensed sequentially on the microplates, filling the wells belonging to the columns numbered from one to ten. The test was performed in triplicate. The microbial inoculum solution was distributed in a volume of 100 µl in the microplate, and positive (inoculum-culture medium) and negative (extract-medium) columns were used. The plates were then incubated at 37˚C in an oven for up to 72 hours. For the reading of the test, visual comparison (turbidity) of the yeast growth occurred in the wells of the different concentrations tested (wells 1 to 10) was performed with its growth in the positive control well. The lowest concentration capable of producing a prominent inhibition (around 50%) of yeast growth relative to the control-positive well was identified as MIC (Minimal Inhibitory Concentration) of the extract for this yeast.

2.5. Statistical Analyses

The analysis was performed using the SPSS Statistics 24.0® program. Data were expressed as mean ± standard deviation of triplicate determinations. The differences between the samples were analyzed by Student's t-test ($p < 0.05$).

3. Results and Discussion

Figure 1 shows the bioactive compounds identified in the aqueous extract of oregano (EAO), ethanolic extract of oregano (EEO), aqueous extract of rosemary (EAA) and ethanolic extract of rosemary (EEA) and their respective concentrations in (mg/g).

In the results of the identification of the bioactive compounds of the species belonging to the family Lamiaceae we can observe the presence of phenolic compounds in the aqueous and ethanolic extracts of *Origanumvulgare* and *Rosmarinus officinalis*. In all extracts analyzed in the present study, rosmarinic

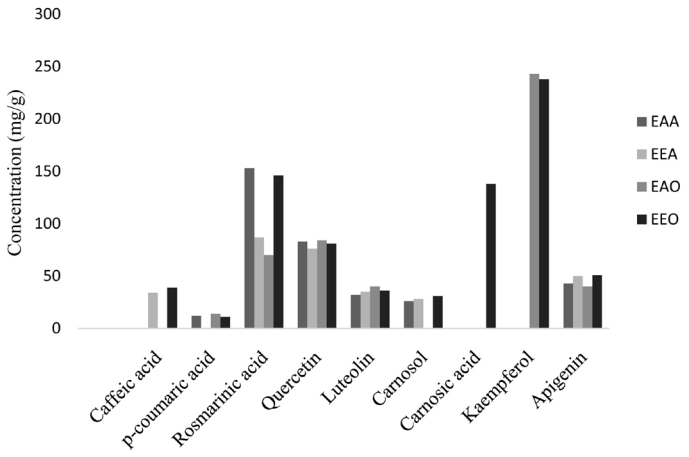

Figure 1. Bioactive compound of oregano and rosemary.

acid, quercetin, luteolin and apigenin were identified. In the aqueous and ethanolic extract of *Origanum vulgare*, *p*-coumaric acid, rosmarinic acid, luteolin, quercetin, Kaempferol and apigenin were identified. In the aqueous and ethanolic extract of rosemary, rosmarinic acid, quercetin, luteolin, apigenna and carnosol were identified. Few studies have been developed to identify the constituents of aqueous and ethanolic extracts, and this identification is important. In the present study, the ethanolic extract of *Origanum vulgare* presented a greater amount of chemical constituents when compared with the other extracts analyzed. Nine phenolic compounds were identified in the ethanolic extract of oregano and six phenolic compounds in the other extracts. The bioactive compounds identified had the following retention times: Caffeic acid 14.86 minutes, p-coumaric acid 25.48 minutes, Rosmarinic acid 53.92 minutes, Quercetin 60.32 minutes, Luteolin 60.68 minutes, Carnosol 67.01 minutes, Carnosic acid 67.30 minutes, Kaempferol 68.08 minutes and Apigenin 69.12 minutes. Kaempferol was the compound found in higher concentration in the aqueous and ethanolic extract of oregano. In the aqueous extract of oregano, the concentration of 243 mg/g and the concentration of 238 mg/g in the oregano ethanol extract were verified. However, this bioactive compound was not found in rosemary extracts. The compound found in the highest concentration in the aqueous extract of rosemary was rosmarinic acid at the concentration of 153 mg/g, whereas in the ethanol extract of rosemary the value of 87 mg/g was found. This compound has been reported in the literature as the main bioactive compound in rosemary [27]. In the ethanol extract of oregano the concentration of rosmarinic acid was 146 mg/g and in the aqueous extract of oregano the value of 70 mg/g. Carnosic acid was found only in the ethanol extract of oregano at the concentration of 138 mg/g. In relation to quercetin, all the extracts present this bioactive compound and the values are close, in the aqueous extract of rosemary was found the value of 83 mg/g, already in the ethanol extract of rosemary the value of 76 mg/g. in the aqueous extracts of oregano the quercetin concentration was 84 mg/g and in the oregano ethanol extract the quercetin concentration was 81 mg/g. Apigenin and luteolin were also found in all extracts. In the aqueous extract of rosemary the concentration of apigenin was 43 mg/g and in the ethanol extract of rosemary the value of 50 mg/g. In the aqueous extract of oregano the value was close to the aqueous extract of rosemary, being found the concentration of 40 mg/g, in the ethanol extract of oregano the value was 51 mg/g. Study reported the presence of apigenin in oregano [28]. Regarding the luteolin concentration the concentration values were also close. In the aqueous extract of rosemary the concentration was 32 mg/g and in the ethanolic extract of rosemary the value of 35 mg/g, in the aqueous extract of oregano the concentration was 40 mg/g in the ethanolic extract of oregano the value of 36 mg/g. Caffeic acid was found in the ethanol extract of rosemary at a concentration of 34 mg/g and in the ethanol extract of oregano at a concentration of 38 mg/g. Carnosol was not identified in the aqueous extract of *Origanum vulgare*, being present in the chromatograms of the other extracts of that study, in the aqueous and ethanolic extracts of rosemary the values were

close, in the aqueous extract of rosemary the concentration was of 26 mg/g in the extracts ethanol concentration of rosemary was 28 mg/g, and in the ethanol extract of oregano the concentration was 31 mg/g. The *p*-coumaric acid was found to be less concentrated in the aqueous and ethanolic extracts of oregano and in the aqueous extract of rosemary, whereas in the ethanol extract of rosemary this compound was not identified. The concentrations found were 12 mg/g in the aqueous extract of rosemary, 14 mg/g in the aqueous extract of oregano and 11 mg/g in the ethanol extract of oregano. Reports show that the types of solvents used to interfere with the amount of bioactive compounds extracted [29] [30] [31]. The aqueous and ethanolic extracts of oregano and rosemary present high content of phenolic compounds, corroding with the results found in the present study [32] [33]. The phenolic compounds found in the plant species of the present study were reported in the research with basil and oregano. Rosmarinic acid was found in basil, a spice belonging to the Lamiaceae family [34] [35]. Carnosic acid, carnosol and rosmarinic acid are the main bioactive compounds present in rosemary [27]. The production of secondary metabolites by plants is related to plant defense mechanisms against pathogens and herbivory [36]. In addition, these bioactive compounds produced by secondary metabolism have shown beneficial effects on human health, aiding in the prevention of diseases due to therapeutic properties [37] [38]. The basil species belonging to the family Lamiaceae, has antioxidant potential [39] [40]. These potentialities are related to the phenolic compounds present in the spice [34] [35]. In relation to the antimicrobial activity of oregano and rosemary, a visual comparison (turbidity) of the yeast growth occurred in the wells of the different concentrations tested (wells 2 to 11) with their growth in the well-positive control. The lowest concentration capable of producing a prominent inhibition (around 50%) of yeast growth relative to the control-positive well was identified as MIC (Minimal Inhibitory Concentration) of the extract for this yeast. To determine the concentration of MFC (Minimal Fungicide Concentration), 10 μL aliquots of the MIC wells were placed in Petri dishes containing the Sabouraud Agar medium in an oven at 37˚C for 48 hours and then the test was read. **Table 1** shows the MIC results of the aqueous and ethanolic extracts of oregano and rosemary on *Candida globosa*, *Candida albicans*, *Cryptococcus laurentii*, *Trichosporon asahii*, *Kodamaea ohmeri*, *Saccharomyces*, *Geotrichum* and *Rhodotorula* sp.

The aqueous and ethanolic extract of oregano and rosemary present antifungal activity against the yeasts tested. Among the extracts used in the present study, the ethanol extract of oregano has the highest antifungal activity. This result suggests that the higher concentration of Kaempferol in extracts of oregano may be responsible for the antimicrobial effect. However, it should be borne in mind that the antifungal potential of this extract may be related to the synergism of the present bioactive compounds. The ethanolic extract of oregano presents similar results for *Candida globosa*, *Candida albicans*, *Cryptococcus laurentii*, *Trichosporon asahii*, *Kodamaea ohmeri* and *Saccharomyces* showing the minimum inhibitory concentration of 1.56 mg/mL. With respect to *Geotrichum*

Table 1. Minimal inhibitory concentration of the aqueous and ethanolic extract of oregano and rosemary on yeasts.

	Minimal Inhibitory Concentration (mg/mL) of the aqueous and ethanolic extract of oregano and rosemary			
Fungus tested	**EAA[1]**	**EEA[2]**	**EAO[3]**	**EEO[4]**
Candida globosa	3.12^{aA}	2.34^{bA}	3.12^{aA}	1.56^{cA}
Candida albicans	3.12^{aA}	2.34^{bA}	3.12^{aA}	1.56^{cA}
Cryptococcus laurentii	3.12^{aA}	2.34^{bA}	3.12^{aA}	1.56^{cA}
Trichosporon asahii	2.34^{aB}	1.56^{bB}	6.25^{cB}	1.56^{bA}
Kodamaea ohmeri	6.25^{aC}	3.12^{bC}	6.25^{aB}	1.56^{bA}
Saccharomyces	3.12^{aA}	1.56^{bD}	3.12^{aA}	1.56^{bA}
Geotrichum	6.25^{aC}	2.34^{bA}	6.25^{aB}	2.34^{bB}
***Rhodotorula* sp.**	1.56^{aD}	1.56^{aD}	1.56^{aC}	0.78^{bC}

[1]Aqueous extract of *R. officinalis*, [2]Ethanolic extract of *R. officinalis*; [3]Aqueous extract of *O. vulgare*, [4]Ethanolic extract of *O. vulgare*; (a, b, c, d) Dissimilar alphabets on the same line indicate significant difference between samples ($p < 0.05$). (A, B, C, D, E, F, G) in the same column indicate a significant difference between the samples ($p < 0.05$).

the minimum inhibitory concentration was 2.34 mg/mL. For *Rhodotorula* sp. the value was 0.78 mg/mL. The results of MIC of the aqueous extract of oregano for *Candida globosa*, *Candida albicans*, *Saccharomyces* and *Cryptococcus laurentii* were 3.12 mg/mL for *Trichosporon asahii*, *Kodamaea ohmeri* and *Geotrichum* were 6.25 mg/mL and for *Rhodotorula* sp. 1.56 mg/mL. In relation to the rosemary extracts, the results of Table 1 show that the ethanol extract of rosemary has higher antifungal activity than the aqueous extract of rosemary. The ethanol extract of rosemary has a minimum inhibitory concentration for *Candida globosa*, *Candida albicans*, *Cryptococcus laurentii*, *Geotrichum*, 2.34 mg/mL. For *Trichosporon asahii*, *Saccharomyces*, *Rhodotorula* sp. the minimum inhibitory concentration was 1.56 mg/mL and for *Kodamaea ohmeri* it was 3.12 mg/mL. The result found in *Rhodotorula* sp. was similar in the ethanol extract of rosemary and aqueous extract of oregano. In relation to the results found in the aqueous extract of rosemary, the lowest concentration was 3.12 mg/mL on *Candida globosa*, *Candida albicans*, *Cryptococcus laurentii*, *Saccharomyces*. The highest concentration was 6.25 mg/mL on *Kodamaea ohmeri* and *Geotrichum*. The results found for MFC are shown in Table 2.

The ethanol extract of oregano also presented lower MFC than the aqueous extract. In the ethanol extract of oregano the MFC for *Candida globosa* was 3.12 mg/mL, for *Cryptococcus laurenti* was 3.65 mg/mL, *Trichosporon asahii* required a concentration of 6.25 mg/mL, *Rhodotorula* sp. 3.65 mg/mL. *Candida albicans*, *Kodamaea ohmeri*, *Saccharomyces* showed a value of 1.56 mg/mL. *Geotrichum* was 2.34 mg/mL. Therefore, it can be MIC and MFC for *Geotrichum*, *Candida albicans*, *Kodamaea ohmeri*, *Saccharomyces* have the same concentrations. Table 2 shows the results obtained from the Minimal Fungicide

Table 2. Minimal fungicide concentration of the aqueous and ethanolic extract of oregano and rosemary in yeasts.

	Minimal Fungicide Concentration (mg/mL) of the aqueous and ethanolic extract of oregano and rosemary			
Fungus tested	**EAA[1]**	**EEA[2]**	**EAO[3]**	**EEO[4]**
Candida globosa	4.68^{aA}	3.12^{bA}	3.65^{cA}	3.12^{bA}
Candida albicans	2.34^{aB}	2.34^{aB}	2.08^{bB}	1.56^{cB}
Cryptococcus laurentii	6.25^{aC}	4.68^{bC}	5.20^{cC}	3.65^{dC}
Trichosporon asahii	6.77^{aD}	6.25^{bD}	6.77^{aD}	6.25^{bD}
Kodamaea ohmeri	6.25^{aC}	6.25^{aD}	4.68^{bE}	1.56^{cB}
Saccharomyces	6.77^{aD}	4.68^{bC}	6.25^{cF}	1.56^{dB}
Geotrichum	4.68^{aA}	3.65^{bE}	2.34^{cG}	2.34^{cE}
***Rhodotorula* sp.**	6.25^{aC}	2.08^{bF}	4.68^{cE}	3.65^{dC}

[1]Aqueous extract of *R. officinalis*, [2]Ethanolic extract of *R. officinalis*; [3]Aqueous extract of *O. vulgare*, [4]Ethanolic extract of *O. vulgare*. (a, b, c, d) Dissimilar alphabets on the same line indicate significant difference between samples ($p < 0.05$). (A, B, C, D, E, F, G) in the same column indicate a significant difference between the samples ($p < 0.05$).

Concentration of the aqueous and ethanolic extract of oregano and rosemary on *Candida globosa*, *Candida albicans*, *Cryptococcus laurentii*, *Trichosporon asahii*, *Kodamaea ohmeri*, *Saccharomyces*, *Geotrichum e Rhodotorula* sp.

Oregano and rosemary extracts present s on the yeasts tested. The aqueous extract of oregano presents MFC of 3.65 mg/mL regarding the *Candida globosa*. The value found for *Candida albicans* was of 2.08 mg/mL. For *Cryptococcus laurentii* was of 5.20 mg/mL. The result was similar for *Kodamaea ohmeri* and *Rhodotorula* sp. presenting value of 4.68 mg/mL. For *Trichosporon asahii* the value found was of 6.77 mg/mL. For *Saccharomyces* was from 6.25 mg/mL and in the *Geotrichum* 2.34 mg/mL. Oregano has been highlighted by antimicrobial and antioxidant properties [41] due to the chemical composition of extracts [28]. Regarding the rosemary extracts, the ethanol extract of rosemary showed higher antifungal activity in the MFC test. Rosemary extracts also present therapeutic potential, especially antimicrobial activities [42] [43] [44] and antioxidant [44] [45].

4. Conclusion

According to the results obtained in this study, it was concluded that the ethanolic extract of oregano presents a greater quantity of phenolic compounds, Caffeic acid, *p*-coumaric acid, Rosmarinic acid, Quercetin, Luteolin, Carnosol, Carnosic acid, Kaempferol and Apigeninand greater antifungal activity against a series of tested yeasts, *Candida globosa*, *Candida albicans*, *Cryptococcus laurentii*, *Trichosporon asahii*, *Kodamaea ohmeri and Saccharomyces*, *Geotrichum* and *Rhodotorula* sp., thus proving that this plant should be carefully evaluated, aiming at a potentiality of this extract for the treatment of fungal diseases. It is possible

to conclude with the result of this study the antifungal potential of oregano, supporting the realization of new studies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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