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Antifungal and Synergistic Effects of the Ethyl Acetate Extract of *Tanacetum vulgare* (L.) Against *Candida albicans*

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Background: With the continued demand for new, effective, and safe endodontic therapies, the aim of this study was assessment of efficiency of the ethyl acetate (EthOAc) extract of *Tanacetum vulgare* (L.) against *Candida albicans*.

Material/Methods: The antifungal effectiveness of the EthOAc extract of *T. vulgare* was determined using the agar disk diffusion method. The inhibition zones induced by the EthOAc extract were compared after 5 minutes, 60 minutes, and 24 hours to those induced by standard solutions (2% chlorhexidine, saturated calcium hydroxide, and 2% sodium hypochlorite). Statistical analysis of the results was performed using the Kruskal-Wallis test and one-way ANOVA.

Results: The inhibition zone of chlorhexidine against *C. albicans* was 30.3–19.3 mm, but in combination with EthOAc extract (100 mg/mL) of *T. vulgare*, this inhibition was from 32.7–30 mm, indicating that this combination exerted a marked synergistic effect against *C. albicans*. The inhibition zone of sodium hypochlorite (69.7–65 mm) was higher than the inhibition zone of EthOAc extract and chlorhexidine. The combination of EthOAc extract with sodium hypochlorite resulted in a loss of antifungal activity. Furthermore, the activity of the EthOAc extract against *C. albicans* was decreased after mixing the extract with dentine at a concentration of 25 mg/50 µL (30.3–20.7 mm). The EthOAc extract did not show a genotoxic effect on lymphocyte cells.

Conclusions: The EthOAc extract of *T. vulgare* may be a useful tool to discover natural bioactive agents that have antifungal activity against *C. albicans* and could be used as endodontic therapies.

MeSH Keywords: Antifungal Agents • *Candida albicans* • Endodontics • Tanacetum

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Background

The purpose of root canal treatment is the removal of diseased tissue, eradication of microorganisms within the root canal and the prevention of recontamination [1]. Instrumentation of root canal should be followed by irrigation, which enables removal of necrotic tissue and microorganisms [2]. Endodontic irrigants are applied to increase the antimicrobial effects of cleaning and shaping in endodontics. Due to close contact with the periodontal tissue, root canal irrigant must be biocompatible.

Sodium hypochlorite is an irrigant which is commonly used in endodontics, because of its ability to destroy a large number of microorganisms, but it is unable to remove the smear layer [3]. It also has undesirable features that include allergic potential, tissue toxicity, and a bad taste [4]. Chlorhexidine (CHX) is another irrigant that confers antimicrobial activity and biocompatibility, but has no tissue dissolving capabilities [5]. Calcium hydroxide saturated in water possesses antimicrobial activity and creates a hard tissue barrier, making it a good choice as an intercanal medication. A small part of the flora survives after treatment with root canal medication and there is evidence of the detected fungi in the root canal system [6]. *Candida albicans* has been observed within root canal and periapical region associated with persistent infections [7,8].

The increased prevalence of resistant strains and the side effects caused by synthetic drugs have challenged researchers to search for herbal therapeutic alternatives. The advantages of plant extracts in healing and treating diseases are well-established. The therapeutic properties of plants are due to their composition being rich in bioactive compounds such as essential oils, polyphenols, flavonoids, or tannins [9]. The essential oils extracted from plants contain terpenes and it is the terpene subclasses, monoterpenes and sesquiterpenes, which have antibacterial, antiseptic, and anti-inflammatory properties [10–12].

Tanacetum vulgare L. is a perennial herb of the *Asteraceae* family [13]. Studies on rats and mice [14,15] have found that *T. vulgare* extracts have anti-inflammatory properties. The volatile oil obtained from parts of *T vulgare* have been shown to have inhibitory effect on germ tube formation in *C. albicans* [16]. Flavonoids present in this plant may be responsible for these biological effects [15,17]. A few studies have reported the antimicrobial activity of herbal root canal irrigants. The aim of this study was to evaluate *T. vulgare* as a potential antifungal agent in the inhibition of *C. albicans* in comparison to sodium hypochlorite, CHX, and calcium hydroxide, and to evaluate its genotoxic effects.

Material and Methods

This paper has been approved by the Ethics Commission (Nr. 2417) and for this study, we used 100 extracted human single-rooted teeth, that were extracted for periodontal and orthodontic reasons. The teeth were stored in 1% Sodium hypochlorite prior to the experiment, and then rinsed and autoclaved at 121°C for 15 minutes. The teeth crowns were removed with a diamond saw (Smart Cut 4002, UKAM, Valencia, CA, USA). After endodontic access, the pulp tissue was removed with barbed broaches. Root canals were enlarged with Endostar E5 (Poldent Co., Warsaw, Poland) according to the manufacturer's instructions to obtain dentine powder. Powdered dentine was suspended in distilled water to 28 mg per 50-µL aliquot.

Medicaments

The medicaments used in this investigation were calcium hydroxide saturated in water, 2% sodium hypochlorite (ChloraXD, Cerkamed, StatowaWola, Poland), and 2% CHX (Gluco-Chex, Cerkamed, StatowaWola, Poland), ethyl acetate (Sigma Aldrich, Switzerland) and dimethyl formamide (DMF; Sigma Aldrich, Switzerland). Sterile water was used as a negative control.

Plant materials

T. vulgare plants were collected in a Kosovo field (Sharri Mountains) in September 2018. A voucher specimen was deposited in the first author's laboratory (AK1/2018). The aerial part of *T. vulgare* was air-dried and finely ground and powdered with a blender. The dried powder was vacuum-packed and stored at –20°C until use. The dried *T. vulgare* powder (10 g) was extracted twice with 100 mL of ethyl acetate (EthOAc) for 48 hours at room temperature. The extracted suspensions were filtered through Whatman No. 1 filter paper and the filtrates were concentrated to dryness using a rotary evaporator, and then stored at –20°C until further use. For the antibacterial activity assays, the extracts were dissolved in N,N-dimethylformamide (DMF) at a concentration of 100 mg/mL and stored at 4°C as stock solutions.

Bacterial strains and inoculum preparation

The antifungal potency of the plant extract, the endodontic irrigant and the combination of plant extract with endodontic irrigants was evaluated using *C. albicans* (ATTC 10231, Liofilchem, Roseto degli Abruzzi, Italy).

The antifungal activities of the Soxhlet extracts of plants and the antifungal activities of endodontic irrigants were analyzed by a disk diffusion assay. The suspension of *C. albicans* was cultured for 48 hours in 1 mL of sterile Sabouraud dextrose broth (Liofilchem, Roseto degli Abruzzi, Italy) at 37°C, then adjusted

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to a turbidity of 0.5 on the McFarland scale (1.5×10^8 cells/mL). This culture was used throughout the experiments. Mueller-Hinton agar (10 mL) was poured into Petri dishes, which were then inoculated with strains of yeast by the addition of 0.1 mL of cell culture media.

Sterile filter paper disks loaded with endodontic irrigants, plant extracts, and a combination of endodontic irrigants and plant extracts (10 mg/mL) were placed on top of Mueller–Hilton agar plates. These plates were incubated at 5°C for 2 hours to permit plant extract diffusion, and then they were incubated at 35°C for 24 hours. Disks that were only impregnated with extract solvent were used as a control. Inhibition zones, considered indicative of antibacterial activity, were measured using Vernier calipers and recorded.

Growth inhibition assay to determinate the antifungal activity of the medicament on dentine powder

Aliquots of 50 µL of dentine powder suspension in water were mixed and incubated with 50 µL of the medicaments in sealed test tubes at 37°C for 1 hour or 24 hours, before the addition of yeast. Control groups consisted of 50 µL of sterile water instead of dentine powder. The total volume of the test and control mixtures was 150 µL. All mixtures were incubated at 37°C. Dentine powder/medicament/yeast suspension were mixed with a sterile pipette twice (before the 1-hour sample) or 3 times (before the 24-hour sample) during the incubation. Samples for bacterial culturing (10 µL per sample) were taken from the experimental and control suspensions at 5 minutes, 1 hour, and 24 hours after the addition of yeast. The plates were observed after 5 minutes, 60 minutes, and 24 hours, according to Bulacio et al. [18] (Figure 1). The results were recorded based on the standard disk method according to the Clinical and Laboratory Standards Institute (CLSI, USA) and the European Committee for Antibiotic Sensitivity Testing (EUCAST).

Genotoxicity assay of the medicaments

An *in vitro* genotoxicity assay was performed in cultured lymphocytes. To prepare the lymphocyte cells, blood (12 mL) was taken from 30 human volunteers. For EthOAc extracts of *T. vulgaris* (0.5 mL), 4 tests were performed. The time duration of the treatment was 72 hours. For the positive control group, we added glyphosate herbicide (36%) and for the negative control group we added no agent. Blood (1 mL) from human volunteers was taken from heparinized venous blood vessels and transferred into 15-mL tubes containing peripheral blood medium (Sigma) for lymphocyte cultivation. Then, the tubes were incubated for 72 hours at 37°C. After 44 hours of incubation, 3 µg/mL of cytochalasin B was added according to method of Fenech and Morley [19–21], which blocks cytokine production but not karyokinesis and produces binuclear cells

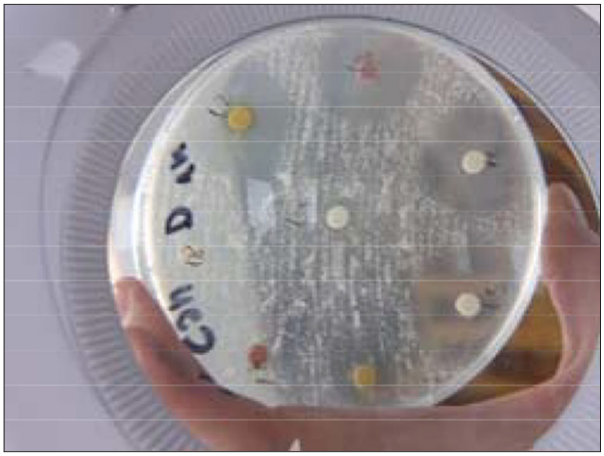

Figure 1. The diameter of inhibition zone of agents used in the study.

within the "parent" cell membranes. All procedures were performed under sterile conditions. After incubation for 72 hours at 37°C, cell cultures were centrifuged for 10 minutes at 1000 rpm. The supernatant was then discarded and the pellet containing lymphocytes was resuspended in 5 mL of hypotonic KCl solution (0.074 M), prewarmed to 37°C, subjecting the cells to hypotonic shock for 10 minutes at room temperature. The cultures were then centrifuged for 10 minutes at 1000 rpm. Again, the supernatant was removed, and the cells were fixed for 20 minutes with fixative solution (3: 1 ratio of absolute ethanol: glacial acetic acid) precooled at 4°C. After centrifuging for 10 minutes at 1000 rpm, the supernatant was removed and further centrifuged until the cell pellet became opaque.

After centrifugation and fixation, the cells were finally resuspended in 1 mL of fresh fixation solution. Six extract preparations were placed onto pre-chilled glass slides, which was sufficient for the counting of 500 LBN-Binuclear lymphocytes and were dried at room temperature for several hours. The preparations were stained with 10% Giemsa solution for 20 minutes, then rinsed with distilled water.

Statistical analysis

Data were processed using the SPSS package 22.0. Testing of quantitative data between groups with a normal distribution was performed with one-way ANOVA, while testing of groups with no normal distribution was performed with the Kruskal-Wallis test. A value of $P<0.05$ was considered statistically significant.

Results

The mean values of fungal growth inhibition produced by EthOAc extract of *T. vulgare*, CHX, sodium hypochlorite and

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Table 1. Influence of EthOAc extract of *Tanacetum vulgare* with and without chemicalsmMedicaments (chlorhexidine, calcium hydroxide, sodium hypochlorite) in the growth of *Candida albicans* without dentine powder (values are in mm).

Number of samples (N=5)	5 min.				60 min.				24 hours			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
EthOAc extract	38.7	0.6	38	39	38.0	1.0	37	39	25.0	0.0	25	25
Ca(OH)2-EthOAc extract	22.3	0.6	22	23	14.7	0.6	14	15	0.0	0.0	0	0
CHX-EthOAc extract	32.7	1.2	32	34	30.7	0.6	30	31	30.0	0.0	30	30
NaOCl-EthOAc extract	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0
$Ca(OH)_2$	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0
CHX	30.3	0.6	30	31	21.3	3.2	19	25	19.3	0.6	19	20
DMF	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0
NaOCl	69.7	0.6	69	70	68.3	0.6	68	69	65.0	0.0	65	65
Kruskal Wallis test	KW=22.8, *P*=0.018				KW=22.8, *P*=0.018				KW=22.9, *P*=0.018			

EthOAc – ethyl acetate; $Ca(OH)_2$ – calcium hydroxide; CHX – chlorhexidine; NAOCl – sodium hypochlorite; DMF – dimethyl formamide; KW – Kruskal Wallis.

Table 2. Inhibitory effect of dentine in the antifungal activity (against *Candida albicans*) of EthOAc extract of *Tanacetum vulgare* with and without chemicals/medicaments (chlorhexidine, calcium hydroxide, sodium hypochlorite), (values are in mm)

Number of samples (N=5)	5 min.				60 min.				24 hours			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
EthOAc extract	30.3	0.6	30	31	24.3	0.6	24	25	20.7	0.6	20	21
Ca(OH)2-EthOAc extract	18.7	0.6	18	19	13.7	1.2	13	15	0.0	0.0	0	0
CHX-EthOAc extract	29.7	1.2	29	31	29.3	0.6	29	30	28.3	0.6	28	29
NaOCl-EthOAc extract	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0
$Ca(OH)_2$	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0
CHX	25.7	0.6	26	26	19.0	1.0	18	20	18.0	1.0	17	19
DMF	0.0	0.0	0	0	0.0	0.0	0	0	0.0	0.0	0	0
NaOCl	60.3	0.6	60	61	55.0	1.0	54	56	40.7	0.6	40	41
Kruskal Wallis test	KW=22.8, *P*=0.018				KW=22.8, *P*=0.018				KW=22.9, *P*=0.018			

EthOAc – ethyl acetate; $Ca(OH)_2$ – calcium hydroxide; CHX – chlorhexidine; NAOCl – sodium hypochlorite; DMF – dimethyl formamide; KW – Kruskal Wallis.

calcium hydroxide after 5 minutes, 60 minutes, and 24 hours are shown in Table 1. All of the experimental groups were statistically different ($P<0.05$) for the microorganism tested. After 5 minutes, when the EthOAc extract was used in combination with sodium hypochlorite in the dentin powder-free solution there was no inhibition zone, whereas when the EthOAc extract was used alone there was a 38.7 mm inhibition zone and when sodium hypochlorite was used alone there was a large inhibition zone of 69.7 mm. At this timepoint, there was a statistically significant difference in the growth of *C. albicans* depending on the material used. After 60 minutes, when the EthOAc extract was used in combination with sodium hypochlorite there was no increase in the inhibition zone, when the extract was used alone there was a decrease in the inhibition zone to 38 mm, and when sodium hypochlorite was used alone there was a decrease in the inhibition zone to 68.3 mm. At this timepoint, there was a statistically significant difference in the growth of *C. albicans* depending on the preparation used ($P=0.018$). After 24 hours, when the EthOAc extract was used in combination with sodium hypochlorite in the dentin powder-free solution there was no inhibition zone, when the extract was used alone there was a 25.0 mm inhibition zone,

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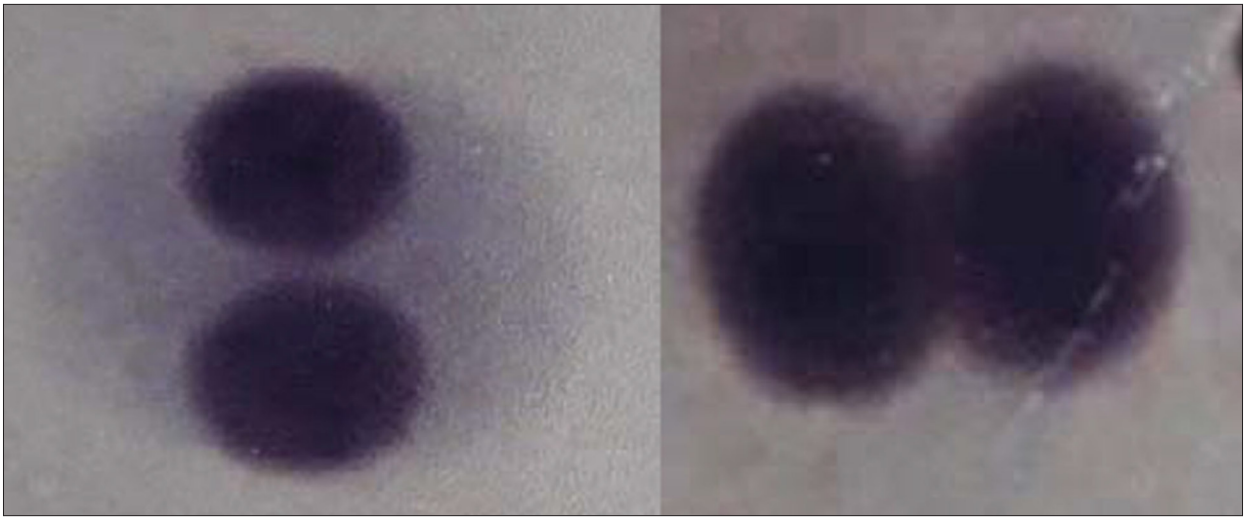

Figure 2. Binuclear lymphocytes without micronucleus, tested with ethyl acetate (EthOAc) extract of *Tanacetum vulgare*.

when sodium hypochlorite was used alone there was a smaller inhibition zone of 65.0 mm, and when the EthOAc extract was used in combination with $Ca(OH)_2$ there was no *C. albicans* inhibition zone. At this timepoint, there was a statistically significant difference in the growth of *C. albicans* depending on the preparation used (*P*=0.018).

With the exception of the combination of EthOAc extract with $Ca(OH)_2$ solution, which showed no *C. albicans* inhibition zone in dentin powder solution, for all other combinations showed slight differences after 60 minutes and 24 hours (Table 2). After 5 minutes planting in the dentin powder, when EthOAc extract was used in combination with sodium hypochlorite there was no inhibition zone, when the extract was used alone there was a 30.3 mm inhibition zone, and when sodium hypochlorite was used alone there was a large inhibition zone of 60.3 mm. There was a statistically significant difference in the growth of *C. albicans* 5 minutes after planting in dentine powder solution depending on the preparation used (*P*=0.018). After 60 minutes, when the herbal EthOAc extract was used in combination with sodium hypochlorite there was no inhibition zone, when the extract was used alone there was a 24.3 mm inhibition zone, and when sodium hypochlorite was used alone there was an inhibition zone of 55.0 mm. There was a statistically significant difference in the growth of *C. albicans* 60 minutes after planting in dentine powder solution depending on the preparation used (*P*=0.018). After 24 hours of planting in the dentin powder solution, when the herbal EthOAc extract was used in combination with sodium hypochlorite there was no inhibition zone, when the extract was used alone there was a 20.7 mm inhibition zone, when sodium hypochlorite was used alone there was a large inhibition zone of 40.7 mm, whereas there was no *C. albicans* inhibition zone when the herbal extract was used in combination with $Ca(OH)_2$. There was a statistically significant difference in the growth of *C. albicans* 24 hours after planting in dentine powder solution depending on the preparation used (*P*=0.018).

Table 3. Genotoxic effects of EthOAc extract of *Tanacetum vulgare* and glyphosate herbicide assessed by induction of micronuclei in human lymphocytes: percentage of micronuclei observed in 500 lymphocytes (no of individuals=30).

Agents	(Mean%±SD)	One-way ANOVA
EthOAc extract	0	
Glyphosate herbicide	3.00±1.42	*P*=0.000027
No agent	0	

EthOAc – ethyl acetate.

The genotoxic effect of EthOAc extracts of *T. vulgare* (Figure 2) and glyphosate herbicide as a control positive agent was evaluated by observing the occurrence of micronuclei in 500 lymphocytes. Testing of the occurrence of micronuclei showed significant difference between glyphosate herbicide and EthOAc extracts (*P*=0.000027) (Table 3).

Discussion

C. albicans was selected for this study because it is the commonly isolated fungus in infected root canals and can survive chemical/mechanical procedures and root canal medications [22,23]. The disk diffusion method was chosen to evaluate the antimicrobial activity of materials, because it is simple and fast method. The priority of this method is that it provides the direct comparison of different materials and indicates their potential to eliminate bacteria within the root

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canal system. Dentine powder was used because it can be easily and rapidly mixed with microorganisms to give a homogenous mixture [24].

Local and systemic antibiotics, as well as prolonged endodontic treatment, contribute in the colonization of the root canal by *C. albicans*. Many herbs family are widely used in treatment of oral diseases such as candidiasis, dental caries and periodontal diseases. Antifungal activity of green propolis has been reported against Candida albicans morphotypes: yeast, pseudohyphae, and hyphae[25]. Turmeric has shown strong antifungal activity against *C. albicans* [26], and EndoPamhas been reported to have potential as an herbal alternative in root canal irrigation [27]. There are in fact many examples in the literature of herb extracts and phytochemicals with antibacterial properties may have potential applications in endodontics [28,29]. Findings have shown that propolis is a promising endodontic drug with comparable antimicrobial efficacy to sodium hypochlorite and CHX against *C. albicans,* even when it is present the smear layer. The effects of propolis on such a resistant microorganism suggest that it could be beneficial in root canal treatment [30].

Most alcohol extracts of *T. vulgare* are reported to have biological effects including anti-inflammatory, antioxidant and antibacterial properties. Furthermore, *in vitro* experiments showed the promising antifungal activity of ethanolic extract of *Praxelisclematidea* (*Asteraceae* family) against *C. albicans* [31,32].

Based on these findings, we decided to use ethyl acetate to extract the metabolites of *T. vulgare* (L.) (*Asteraceae* family), and to test this organic extract for antifungal activity against *C. albicans*. The EthOAc extract from *T. vulgaris* demonstrated the strong antifungal activity against *C. albicans.* We also used different medicaments as standards and these included CHX 2%, sodium hypochlorite 2% and a saturated solution of calcium hydroxide. Sena et al. [33] evaluated the effect of liquid and gel forms of 2% CHX against biofilms of *C. albicans* on cellulose nitrate membranes. They found that a 2% solution of CHX had high antifungal activity against *C. albicans*. The contact time required to achieve negative cultures ranged between 30 seconds to 30 minutes. In our experiment, we found that EthOAc extract (38 mm inhibition zone) had higher antifungal activity against *C. albicans* than 2% CHX (30 mm), which is currently used in endodontic therapy. The antifungal activity of the combination of EthOAc and CHX (32 mm) was higher than that of CHX alone (30 mm).

Studies have shown that sodium hypochlorite is a potent killing agent of *C. albicans*, whereas this organism is resistant to calcium hydroxide. According to Mohammadi et al., saturated calcium hydroxide showed no antifungal effects, while the best antifungal effects were shown by sodium hypochlorite and 2% CHX [34], and 0.5–5.25% sodium hypochlorite completely eliminated *C. albicans* in a study by Vianna et al. [35]. Waltimo et al. [36] showed that both 5% and 0.5% concentrations of sodium hypochlorite caused complete eradication of *C. albicans* cells after 30 second. In our study, 2% sodium hypochlorite had higher antifungal activity against *C. albicans* (70 mm inhibition zone) compared with all other medicaments that we have used (EthOAc extract, 2% CHX and a saturated solution of calcium hydroxide). However, when a mixture of EthOAc extract and 2% sodium hypochlorite was applied, the antifungal activity of extracts and sodium hypochlorite was lost. We postulate that the sodium hypochlorite may be "spent" by oxidating the bioactive compounds which are present in the EthOAc extract and that this may be the reason why the antifungal activity of sodium hypochlorite and the EthOAc extract is lost.

Studies by Ballal et al. [37] and Ferguson et al. [38] demonstrated that calcium hydroxide had no detectable antifungal effect. It has been reported that *C. albicans* cells are highly resistant to calcium hydroxide and that aqueous calcium hydroxide had no antifungal activity when in direct contact with *C. albicans* cells [38].Turk et al. and Pacios et al. reported that *Enterococcus faecalis* and *C. albicans* were not inhibited by calcium hydroxide mixed with distilled water [39,40]. Our results were consistent with these findings. In our study, the saturated solution of calcium hydroxide did not show any antifungal activity against *C. albicans*. Furthermore, the mixture of EthOAc extract with calcium hydroxide solution had lower (22.3 mm) antifungal activity against *C. albicans* compared with the individual antifungal activity of EthOAc extract (38 mm). It was therefore clear that calcium hydroxide decreased the antifungal activity of EthOAc extract of *T. vulgare* against *C. albicans*.

Dentine powder had an inhibitory effect on all tested medicaments. This was similar with the results of Haapasalo et al. [24]. The experiment with dentine powder resulted to be an efficient method with which, it may be evaluated the interactions between root canal agents, dentine and microorganisms [24]. Haapasalo et al. (2000) investigated the inhibitory effect of dentine against some commonly used root canal irrigants. The tested medicaments were calcium hydroxide statured in water, 1% sodium hypochlorite, 0.5% and 0.05% chlorhexidine acetate, and 2–4% and 0.2–0.4% iodine potassium iodide. As a microbe was tested *E. faecalis.* The aforementioned authors reported that the dentine powder had an inhibitory effect on all tested medicaments against *E. faecalis*. The inhibitory effect was dependent on the concentration of the medicament and the duration of preincubation time with dentine powder before the addition of bacteria. Based on this, we tested the antifungal activity of EthOAc extract of *T. vulgaris* and other medicaments after mixing with dentine powder.

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We found that the dentine powder also had an inhibitory effect on all of the tested medicaments: EthOAc extract, CHX, and sodium hypochlorite.

Based on our preliminary investigations, the results of the agar-diffusion tests showed that 2% CHX and 2% sodium hypochlorite solutions from containers opened several days previously produced smaller inhibition zones than solutions from newly opened containers. This was the reason why we tested the antifungal activity of the EthOAc extract and medicaments after 1 hour and 24 hours of the containers being opened, against *C. albicans*. The antifungal activity of EthOAc extract decreased with time, with inhibition zones decreasing from 38.7 mm (5 minutes) to 25 mm (after 24 hours). The same trend was seen for CHX and sodium hypochlorite. The saturated solution of calcium hydroxide did not show antifungal activity in any of our experimental tests.

In an *in vitro* study of Roselli et al. [41] the investigated subspecies of *T. vulgare* showed no variability in chemical composition and could be classified into belonging to the eudesmanolide chemotype. According to the same study, all components showed cytotoxic activity against cultured cancer and healthy cell lines. In contrast, we found that EthOAc extracts of *T. vulgare* did not show any genotoxic effects in lymphocyte cells *in vitro*. Glyphosate herbicide (36%, 0.5 mL) was used as a positive control for this experiment and showed genotoxic effect, where the average of micronuclei formed was 15, or 3.00±1.42% of the 500 lymphocytes observed.

Because endodontic infections involve diverse microbes, drugs that are effective against a microorganism *in vitro* may not be effective *in vivo*. Therefore, studies *in vivo* are required to confirm the effects of calcium hydroxide, CHX, sodium hypochlorite, and EthOAc extract of *T. vulgare* against *C. albicans*. Further studies on suitable medicinal plants, their use and dosage are required to gain more information about their toxicity and possible side effects.

Conclusions

During this research, we evaluated the antifungal activity of the EthOAc extract of *T. vulgare* against *C. albicans*. The EthOAc extract from *T. vulgare* has been shown to have high antifungal activity compared with commercial irrigant such as CHX, and may be used as an effective, safe and natural source of antifungal agent. This EthOAc extract in combination with the medicament CHX showed an additive effect on the antifungal activity against *C. albicans*. The results of the present investigation will contribute to the development of an alternative phytotherapeutic agent to treat infection with *C. albicans*.

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