

# Anti-*Trichomonas vaginalis* activities of some Lamiaceae plants

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**ABSTRACT:** Trichomoniasis is a sexually transmitted infection caused by the *Trichomonas vaginalis*, non-viral protozoan, affecting the world. Metronidazole is one of the effective treatments approved by the FDA. Natural alternative approaches are required due to developed of resistance and drug intolerance in long-term treatment. The Lamiaceae, with 250 genera and 3000 species, are one of the largest family based on the taxon numbers. *Thymus*, *Salvia*, and *Phlomis* are also the largest genus of the family. The most distinctive feature of the species is active compounds of antiprotozoal activities. In present study *Phlomis armeniaca* Willd., *Phlomis pungens* Willd. var. *pungens*, *Phlomis sieheana* Rech. Fil., *Thymus haussknechtii* Velen., *Thymus kotschyanus* Boiss. & Hohen. var. *kotschyanus*, *Stachys lavandulifolia* Vahl subsp. *lavandulifolia* and *Salvia verticillata* L. subsp. *verticillata* L. had investigated anti-*Trichomonas vaginalis* activity. Results indicated that *Thymus haussknechtii* was found anti-trichomonal activity in 250 and 125 µg/ml concentrations at the end of 16 hours. The inhibition of *Salvia verticillata* subsp. *verticillata* and *Phlomis sieheana*. were started after 24 hours, and anti-trichomonal activity was detected 70% and 90% at the end of 48 hours, respectively.

**KEYWORDS:** Lamiaceae; *Phlomis*; *Salvia*; *Thymus*; *Trichomonas vaginalis*.

## 1. INTRODUCTION

Sexually transmitted diseases (STDs) continue to be an important global problem despite many successful prevention and control efforts programs. STDs' most important etiological factors include bacteria, viruses, and protozoa. Trichomoniasis, caused by *Trichomonas vaginalis* (TV) is a parasitic protozoan is an important non-viral but STDs that affects both men and women [1]. According to the 2012 report of the World Health Organization, it was estimated that 276,4 million cases worldwide were infected with TV in a year, and most of them were women [2]. TV infects the squamous epithelium of the genital tract and causes several symptoms including vaginitis, cervicitis, and urethritis. The infection is highly prevalent, often asymptomatic, and easily transferable between sex partners. Metronidazole is a member of the nitroimidazole group, a major FDA-approved medication that have been used as the only active drug since 1960 in treatment of trichomoniasis [3]. Treatment of the disease is generally successful, but recent studies show that many clinical cases are resistant to the drug and side effects are reported increasingly. Also, the main disadvantage of the drug is low absorption. Therefore, new therapeutic alternatives are urgently required to control trichomoniasis.

Natural products, especially plant-derived, attract the scientific world's attention due to their easy accessibility and cheapness, as well as their traditional uses [4]. Lamiaceae family includes 3000 species and 250 genera worldwide. The family is the third largest family with 48 genera and 782 taxa, and almost half of the species are endemic in Turkey [5]. The plants are taste regulators and flavorings with aromatic properties with essential oils. It contains many economically herbal-based products, medicinal, fragrances, food, and ornamental plants. Lamiaceae family including *Thymus* L., *Salvia* L., and *Phlomis* L. have the largest genera with 43,107, and 53 taxon numbers, respectively [6]. They have therapeutic uses in medicine due to their antioxidant and antibacterial properties. It is known that these effects are related to the density of phenolic compounds [7]. *Thymus haussknechtii* Velen. is an endemic species of *Thymus* naturally grown in the Eastern Anatolia region (especially, Erzincan-Sivas province) in Türkiye [8,9]. *Salvia* genus has medicinal and

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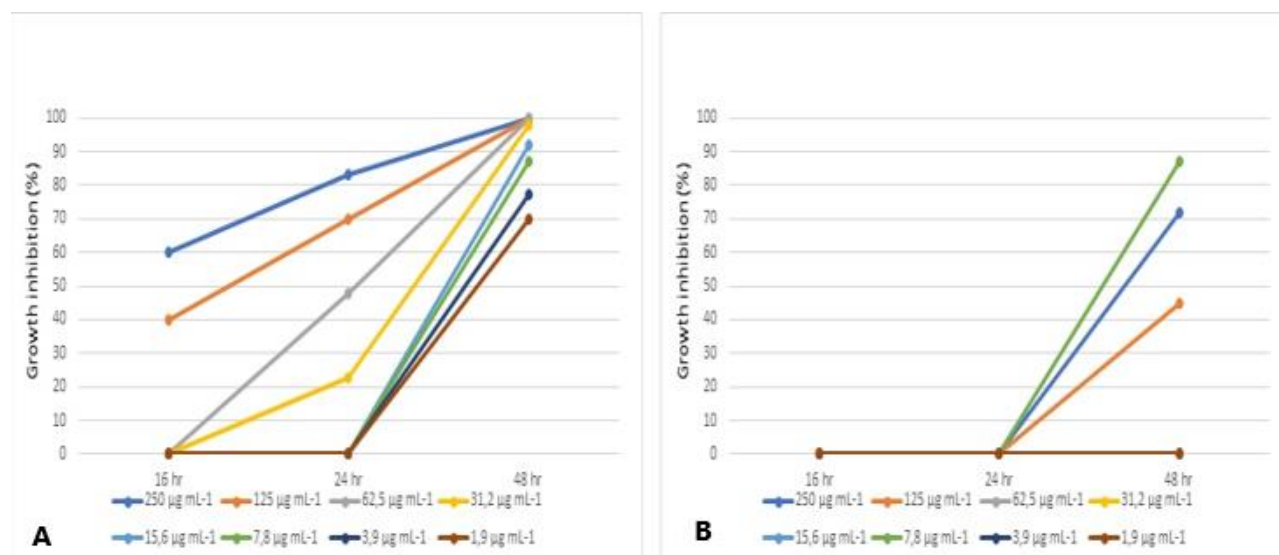
aromatic plants used in traditional medicine and cosmetic industry. Recent studies have shown that *Thymus*, *Salvia*, and *Phlomis* species have various pharmacological and biological properties such as antibacterial, antifungal, antiviral, insecticidal, antioxidant, and antiprotozoal activities. [10-16]. The biological activity activities of these phytochemicals have been reported to be related to phenolics, flavonoids, terpenoids, saponins, and tannins [17].

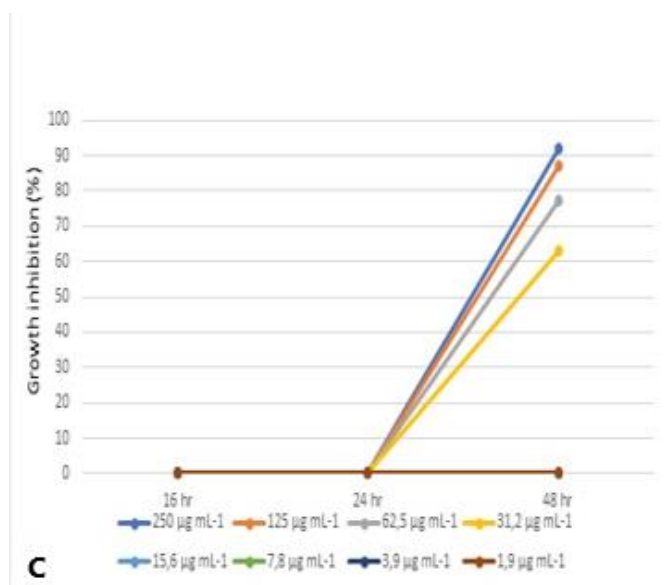
In present study, *in vitro* activity of *Phlomis armeniaca* Willd., *Phlomis pungens* Willd. var. *pungens* Willd., *Phlomis sieheana* Rech. Fil., *Thymus haussknechtii* Velen., *Thymus kotschyanus* Boiss. et Hohen. var. *kotschyanus* Boiss. et Hohen., *Stachys lavandulifolia* Vahl var. *lavandulifolia* Vahl and *Salvia verticillata* L. subsp. *verticillata* L. extracts were investigated against *T. vaginalis* trophozoites for the first time.

## 2. RESULTS

As a result of the study, anti-trichomonal activity was detected only in *Thymus haussknechtii* (TH) at 250 and 125 µg/ml concentrations after incubation for 16 h. Inhibition started after 24 hours and approximately 70% and 90% inhibition were found for *Salvia verticillata* subsp *verticillata* and *Phlomis sieheana*, after 48 hours, respectively.

The minimum inhibitory concentration (MIC) value of TH compound was found at 62,5 µg/ml at the end of 48 h, while 15,6 µg/ml was for metronidazole. The 48 h Minimal lethal concentrations (MLC) value for TH was 62,5 µg/ml. The DMSO control was found to be inactive against trophozoites. *Phlomis armeniaca*, *Phlomis pungens*, *Thymus kotschyanus*, and *Stachys lavandulifolia* compounds did not have anti-trichomonal activity. Figure 1 represents the growth inhibition rates (GI, %) of TH, SV, and PS compounds at 16, 24, and 48 h.





**Figure 1.** Comparison of growth inhibition (%) of *Trichomonas vaginalis* trophozoites in the presence of different concentrations of A) TH, B) SV, and C) PS extracts.

### 3. DISCUSSION

Metronidazole has been the main therapeutic option for the treatment of trichomoniasis for many years. However, the search for new and natural products has gained great importance due to their severe side effects and resistance to TV strains. Plant species from the Astereaceae, Myrtaceae and Lamiaceae families are used to cure many diseases. The Lamiaceae plant family, also known as Labiatae, are herbs or shrubs that often have aromatic scents and are grown for medicinal, perfumery, culinary, and ornamental purposes. Recent studies have shown that these plants have antimicrobial, antiviral, anti-inflammatory, antioxidant, spasmolytic, antidiabetic, and antibacterial effects. However, there are few studies on the antiprotozoal effects of these compounds. Previous studies emphasized that some Lamiaceae families contain alkaloids, isoflavonoid glycosides, essential oils, lipids, saponins, and sesquiterpene lactones and exhibit anti-*Trichomonas* activity [18,19]. In addition, their phenolic and polyphenolic constituents are also used for medicinal purposes [20].

In the present study, the in vitro antitrichomonal activity of *Tyhmus*, *Salvia*, *Phlomis* and *Stachys*, subspecies of the Lamiaceae family, was investigated for the first time in Türkiye. The antiprotozoal activity of *Thymus vulgaris* was demonstrated in vitro against *Trichomonas vaginalis*, *Giardia lamblia* and *Entamoeba histolytica* trophozoites. The results showed that concentrations of 300 µg/ml inhibited the growth of *Trichomonas vaginalis* and *Giardia lamblia* trophozoites. Fractions containing ursolic acid were more effective for trophozoites [21]. According to a study conducted in Iran, *Thymus vulgaris* was effective against trophozoites of *Entamoeba histolytica* [22]. In this study, TH extracts were evaluated against TV trophozoites and were found to be similarly active to metronidazole at concentrations of 250 and 125 µg/ml at the end of 48 hours.

Nowadays, numerous studies have been conducted on the antiprotozoal activity of Lamiaceae plants. In one study, the essential oils of these plants were found to decrease the percentage of vitality and mobility of TV trophozoites [23]. In another study evaluating the anti-*Trichomonas* activity of plants with hydroalcoholic extracts, it was found that trophozoites did not grow at concentrations of *Salvia officinalis* 2, 2.5, 4, 5, 8, and 10 mg/ml and *Menthe piperita* 4, 5, 8, and 10 mg/ml. The ingredients were found to be effective against TV growth, and it was concluded that these plants could be considered as alternative medicines for trichomoniasis [24]. The essential oils of *Lavandula angustifolia* and *L. intermedia* and *Zataria multiflora* were found to eliminate viable trophozoites of TV [25]. The anti-trichomonas activity of *Mosla chinensis* was found to be effective in vitro [26]. In a study to evaluate the antibacterial and antiprotozoal activity of *Hyptis albida*, the aerial parts were found to have antitrichomonal activity [27].

In an earlier study, *Stachys lavandulifolia*, collected from Khostan trees, have been detected anti-trichomonal activity [18-20]. For present research, *Stachys lavandulifolia* var. *lavandulifolia* was collected from the Elazığ province, but ineffective against TV trophozoites in vitro. This difference may be due to the environmental conditions of the plant and the secondary metabolites varieties.

The antiprotozoal activity of *Phlomis lycia* D. Don. was evaluated. IC<sub>50</sub> values of water and chloroform extracts were found 139.19 and 748.58 µg/ml, respectively [28]. In the current study, water, chloroform, and n-hexane extracts of *Phlomis* species were detected effective at different concentrations. When compared with the literature, water extract is more effective than chloroform. Antiprotozoal activities of *Salvia tomentosa* Mill., *S. sclarea* Clary, *S. dichroantha* Stapf., *S. hydrangea* DC. ex. Benth., *S. miltiorrhiza* Bunge, *S. repens* Burch. ex Benth., *S. spathacea* Greene., *S. lavandulifolia* Vahl., and *S. officinalis* L. plants extract were evaluated and IC<sub>50</sub> values were calculated. These extracts have shown that effective inhibitory activity against many protozoa. However, *S. hydrangea* was found to be ineffective against *Leishmania* species. In addition, the essential oil of *S. lavandulifolia* was not effective against *Trypanosoma brucei* [16]. In many studies, the antiprotozoal activity of *S. polystachya* Cav., *S. hydrangea*, *S. circinata* Cav., and *S. sahendica* Boiss. extracts and their substances were found as active compounds against *Entamoeba histolytica* and others [29-31]. Antiprotozoal activities of *Phlomis kurdica* Rech. fil., *P. leucophracta* P. H. Davis & Hub.-Mor. and *P. russeliana* (Sims) Lag. ex Benth. were investigated. The IC<sub>50</sub> value of *P. kurdica* hexane extract was 2.7 µg/ml. Antiprotozoal activity was found of *P. leucophracta* and *P. russeliana* chloroform extract [32]. Our study showed that after 48 hours, *Salvia verticillata* subsp. *verticillata* and *Phlomis sieheana* were inhibited trophozoites of TV by 70% and 90%, respectively.

#### 4. CONCLUSION

According to our hypothesis, anti-*Trichomonas vaginalis* activity is expected in different plants of *Thymus*, *Phlomis*, *Salvia*, and *Stachys* species based on secondary metabolites. As a result, only *Thymus* and *Phlomis* species were effective against TV trophozoites. The lack of activity in other plants is thought to be the change in secondary metabolites depending on the collection time and growing conditions. Therefore, our study provides useful data, and similar studies may shed light on new alternative approaches to treating trichomoniasis.

#### 5. MATERIALS AND METHODS

##### 5.1. Plant extractions

The aerial parts of *Phlomis armeniaca* Willd., *Phlomis pungens* Willd. var. *pungens*, *Phlomis sieheana* Rech. Fil., *Thymus haussknechtii* Velen., *Thymus kotschyanus* Boiss. & Hohen. var. *kotschyanus*, *Stachys lavandulifolia* Vahl subsp. *lavandulifolia* and *Salvia verticillata* L. subsp. *verticillata* were collected in July 2018 from Elazığ, Türkiye. Ugur Cakilcioglu and Bijen Kivcak identified the plants. Voucher specimens of *Phlomis armeniaca* (PA) (1636), *Phlomis pungens* var. *pungens* (PP) (1649), *Phlomis sieheana* (PS) (1650), *Thymus haussknechtii* (TH) (1637), *Thymus kotschyanus* var. *kotschyanus* (TK) (1638), *Stachys lavandulifolia* subsp. *lavandulifolia* (SL) (1651), and *Salvia verticillata* L. subsp. *verticillata* (SV) (1648) are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, İzmir, Türkiye.

40 g of powdered plant material was extracted with methanol (400 mL) in a Soxhlet apparatus for 8 h. The solvents were evaporated to the dryness at 40 °C. Extracts were stored at +4 °C in dark conditions until analyses. The extraction yields of methanol extracts of PA, PP, PS, TH, TK, SL, and SV were calculated as 14.46%, 21.32%, 10.83%, 12.46%, 14.84%, 18.64%, and 16.02%, respectively.

##### 5.2. Trypticase yeast extract maltose (TYM) medium

The trophozoites of *T. vaginalis* were cultivated in TYM medium (1 g L-cysteine HCl, 0.2 g L-ascorbic acid, 0.8 g K<sub>2</sub>HPO<sub>4</sub>, 0.8 g K<sub>2</sub>HPO<sub>4</sub>, 20 g trypticase, 10 g yeast extract, 5 g maltose, 900 ml distilled water) without agar. Medium pH was adjusted to 6,5 and autoclaved at 121 °C for 15 minutes. After cooling of media to 50 °C and supplemented with %10 heat-inactivated fetal bovine serum. Finally, the antibiotics were added as in the following concentrations: 100 U/ml penicillin, 0,08 mg/ml gentamicin and 0,044 mg/ml fluconazole. The media were dispensed into 10 ml screw-capped sterile tubes and stored at -20 °C until use. The media were incubated at 37 °C before cultivations.

### 5.3. Cultivation of *T. vaginalis*

The cryopreserved trophozoites of *T. vaginalis* (ADUTR011) were previously stored in liquid nitrogen at Aydin Adnan Menderes University Faculty of Medicine, Department of Parasitology. The isolates were thawed with TYM medium and incubated at 37°C. The trophozoites of *T. vaginalis* were checked for the logarithmic phase once in three days with a light microscope, and sub-cultures were prepared subsequently. The media with logarithmic growth of parasite were centrifuged at 600g for 5 min, and the final concentration was adjusted to 10<sup>5</sup> cells/ml with a cell counting chamber.

### 5.4. Anti-trichomonal activity

Seven plant species were tested in vitro against *T. vaginalis* trophozoites. All plant compounds were diluted in dimethyl sulfoxide (DMSO) into a final concentration of less than 1%. The 96-well microtiter plates were filled with TYM media containing 1×10<sup>5</sup> trophozoites/ml. The plant compounds were added to the wells at the concentrations of 250 to 1,9 µg/ml. Plates were incubated at 37°C for 16, 24, and 48 hours. Following the incubation periods, an aliquot of trophozoites and trypan blue dye (0.4%) (1:1, v/v) mix was screened in the cell counting chamber to determine the viability of *T. vaginalis* trophozoites. Three controls were used in each of the assays: negative control with viable trophozoites, solvent DMSO control, and a positive control containing metronidazole (MTZ) at 100 µM (Sigma-Aldrich, St. Louis, Missouri, USA). All the assays were repeated three times for each of the plant compounds.

The MIC values were calculated as the final dilution in which no motile *T. vaginalis* trophozoites were observed. The growth of *T. vaginalis* was confirmed with MLC, and was checked after 16, 24, and 48 hours incubation periods.

The growth inhibition percentage was calculated using the following formula: “(A-B) / A × 100”, A is the average number of the control group’s trophozoites, and B is the average number of trophozoites in the test group.

### 5.5. Cell Counting with a hemocytometer

Viability determination of trophozoites was evaluated by hemocytometry at the end of 16, 24, and 48 hours. After the incubation periods, samples were taken from each well of the 96-well plates and stained with trypan blue dye under the microscope. The number of viable trophozoites in one ml medium was calculated as previously reported [33].

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