EVALUATION OF ANTI-TRICHOMONASE ACTIVITIES OF
METHANOL EXTRACT OF *Hypericum scabrum* L. ⁽¹⁾*Hypericum scabrum* L. METHANOL EKSTRAKTININ ANTI-
TRICHOMONAS AKTİVİTESİNİN DEĞERLENDİRİLMESİNecati OZPINAR¹, Hulya OZPINAR², Nuraniye ERUYGUR³, Tuğba KAYA⁴¹ Hatay Mustafa Kemal University, Faculty of Health Sciences, Antakya / Turkey² Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas / Turkey³ Selcuk University, faculty of Pharmacy, Department of Pharmacognosy, Konya / Turkey⁴ Hatay Mustafa Kemal University, Faculty of Medicine, Department of Parasitology Antakya / TurkeyORCID ID: 0000-0002-7317-885X¹, 0000-0001-8154-0874², 0000-0002-4674-7009³,
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Öz: Amaç: Bu çalışmada, *Hypericum scabrum*'un metronidazole duyarlı ve dirençli *Trichomonas vaginalis*'ler üzerine etkisinin araştırılması amaçlandı. **Yöntem:** *Hypericum scabrum* örnekleri Sivas-Ankara karayolundan toplandı. Toprak üstü kısımları kurutularak masserasyon yöntemi ile metanol ekstaksiyonu yapıldı. Elde edilen ekstraktlara GC-MS analizi yapılarak olası etken maddeler tespit edildi. *Trichomonas vaginalis* suşarı 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.6 mg/mL, 0.3 mg/mL, 0.15 mg/mL konsantrasyonlarda bitki ekstraktları ile muamele edildi. **Bulgular:** Metronidazol dirençli *Trichomonas vaginalis* suşalarının Minimum İnhibisyon Konsantrasyonu 2 saatlik bir muamele sonunda 2.5 mg/mL, 4 saatlik muamele sonunda ise 1.25 mg/mL lik konsantrasyonlarda olduğu görüldü. Bu very metronidazole duyarlı suşlarda ise 2 saat sonunda 2.5 mg/mL, 4 saat sonunda ise <0.15 mg/mL olarak tespit edildi. **Sonuç:** *Hypericum scabrum*'un özellikle metronidazole dirençli suşlar üzerindeki etkisi oldukça önemlidir. Bulgularımız bu bitkinin yeni ilaç aktif bileşik izolasyonu için kullanılabileceği düşündürmektedir.

Anahtar Kelimeler: *Hypericum Scabrum*, Anti-*Trichomonas* Aktivite, GC-MS

Abstract: Aim: The objective of the study to evaluate the methanolic extracts of aerial parts of *Hypericum scabrum* L. for in vitro anti-trichomonase activity. **Methods:** Plant material were collected during the flowering period from natural populations in Sivas province of Turkey. Collected locality is: B6 Sivas: Sivas-Ankara road. The extracts obtained were analyzed by GC-MS to identify possible active substances. *Trichomonas vaginalis* strain was exposed with plant extracts at concentrations of 5 mg / mL, 2.5 mg / mL, 1.25 mg / mL, 0.6 mg / mL, 0.3 mg / mL, 0.15 mg / mL. **Result:** The minimum inhibitory concentration (MIC) was found to be 2.5 mg / mL at the end of 2 hours, 1.25 mg/mL at the end of 4 hours, and 0.6 mg/mL at the end of 24 hours after resistant *T. vaginalis* strains exposed to *H. scabrum* methanol extract. When susceptible strains are to investigated, at the end of 2 hours the MIC value of 2.5 mg/mL is the lowest dose to the study, at 4 hours later even at the concentration of 0.15 mg/mL, no live parasite is found. **Conclusion:** The obtained results suggest that the finding can used to further bio-assay guided active compound isolation from this plant as promising resource.

Key Words: *Hypericum Scabrum*, Anti-*Trichomonas* Activity, GC-MS

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INTRODUCTION

The genus *Hypericum* (Hypericaceae) is represented by more than 400 species throughout the world, is mainly distributed in the Mediterranean and the Near East area (Simpson, 2006:122). In Turkey, *Hypericum* comprises of 96 species, in which half of them are endemic (Guner et al., 2000:2-28; Tekin, 2017:143-152). *Hypericum* species are known for the local name of “sarı kantaron” and “binbir delik out”, have been used for treatment of wound healing, sedative, antiulcer, antidiabetic, antispasmodic and antiseptic in Turkish folk medicine (Bingol et al., 2011:86-90). Phytochemical investigations on Hypericaceae revealed that they contain naphthodiantrones (hypericin and pseudohypericin), acylphloroglucinol derivatives (hyperforin and adhyperforin), flavonoids, tannins, and essential oils (Maggi et al., 2004:702-711; Zorzetto et al., 2015:95-109). The major components in fatty acids of *H. scabrum* L. were α -linolenic, linoleic and oleic acid (Ozen and Bashan, 2003:723-726). It was reported that α -Pinene, β -Pinene, spathulenol, *p*-cymene, acetophenone, and carvacrol were the main constituents of the essential oil of *H. scabrum* (Tabanca et al., 2015:62-72). Studies have shown that *Hypericum* species have many biological activities such as antioxidant (Boga et al., 2016:249-257; Mandrone et al., 2015:402-408; Silva et al., 2005:157-167), antimicrobial (Boga et

al., 2016:249-257), cytotoxicity (Tala et al., 2015:149-155) and enzyme inhibition activity (Mandrone et al., 2015:402-408). In spite of many biological activity study on *Hypericum* species, there was little study on *H. scabrum* L.

Trichomoniasis is a most common protozoan infectious disease of the urogenital tract of humans caused by *Trichomonas vaginalis* (*T. vaginalis*), which is the reason why the rate of illness is high in women who have reached sexual maturity. *T. vaginalis*, an anaerobic protozoan that causes trichomoniasis in humans, moves by itself with a whip and waving membrane. Trichomonal infection has a cosmopolitan distribution and is detected in all racial groups and socioeconomic layers. Approximately 333 million new Sexually Transmitted Diseases (STDs) occur annually in the world, of which 170 million are *T. vaginalis* infections (WHO, 2012). Metronidazole is the only drug for therapy of this disease approved by FDA. However, metronidazole has been reported for the resistance by *T. vaginalis* (Kirkcaldy et al., 2012:939; Schwebke and Barrientes, 2006:4209-4210; Snipes et al., 2000:3004-3009).

The aim of the present study focuses on the investigation of anti-*Trichomonase* activity of *H. scabrum* methanol extract.

MATERIAL and METHODS

GC-MS analysis was performed by GRUM-LAB (Giresun University Center Research Laboratory, Application and Research Center)

Plant Materials

Plant material were collected during the flowering period from natural populations in Sivas province of Turkey. Collected locality is: B6 Sivas: Sivas-Ankara road, 25 km.

Preparation of the Extracts

The dried plant materials were powdered using a grinder. The extraction was done at room temperature. 10 g of dried and grounded herbs were extracted with methanol (250 mL×4) for 24 h with intermittent shaking. Then mixture were filtered through a filter paper (Whatman, No.1). The filtrates combined together and concentrated under vacuum on a rotary evaporator (Buchi R-100 equipped with Vacuum Pump V-300 and Control unit I-300) at 40°C and stored at -20°C for further processing. The yields of crude methanol extracts of *H. scabrum* was obtained as 18.9 %.

Anti-Trichomonase Vaginalis Activity

The metronidazole-resistant *T. vaginalis* ATCC 50143 and the metronidazole-sensitive strain, *T. vaginalis* ATCC50148 strain

(obtained by Amerikan Type Culture Collection (ATCC) were used in this study.

Culture of *Trichomonase Vaginalis*

Trichomonas Broth (TB, liofilchem, 610061) medium was purchased commercially and was prepared according to the manufacturer's instructions. After preparation of the TB, it was distributed among the experimental tubes and placed in the autoclave at 121°C for 15 mins, then cooled to 37°C, and 10% inactive horse serum (Sigma, 1234598765) was added to the medium. The *T. vaginalis* strains were added to the TB medium and incubated for 3 days at 37°C under anaerobic conditions.

In Vitro Anti-Trichomonas Assay

The Minimum Lethal Dose (MLD) of the metronidazole-sensitive strain, *T. vaginalis* ATCC50148, and the metronidazole-resistant strain, *T. vaginalis* ATCC50143 against metronidazole was tested in comparison with the plant extract. For this purpose, 96-well plates were used. The *T. vaginalis* strains produced from seeding in the TB medium at 37°C were incubated in metronidazole (Sigma, 1711544348111) concentrations of 400 µM, 200 µM, 100 µM, 50 µM, 25 µM, 12,5 µM, 0,6 µM and 0,3 µM. and plant extract concentration 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.6 mg/mL, 0.3 mg/mL, 0.15 mg/mL After 2-4-24 h, the incubated live protozoa were checked on a Thoma slide for flagellated and

undulating membrane movement and were counted in a 1% eosin solution. A dose where no live parasites were found was determined microscopically and evaluated as MLD.

RESULTS

Chemical Composition by GC-MS Analysis

The chemical compositions of methanol extract of *Hypericum scabrum* aerial parts have

been determined by GC-MS (Table 1). The methanol extract was found to have a higher component content. While the most abundant components are palmitic acid (8.78 %), benzoic acid (2.97%), and -1-carbaldehyde (2.01%) for methanol extract (Table 1). Among the compounds, the 4H-Pyran-4-one are important for the biosynthesis of the hypericin that are main compound of *Hypericum* species.

Table 1. Chemical Compositions of Methanol Extracts of *Hypericum scabrum*

Components	RT	Methanol (%)
Ethanone	14.268	0.65
Benzoic acid	15.212	2.97
4H-Pyran-4-one	17.054	1.37
Isopropyl phenyl ketone	27.542	1.74
Naphthalene	28.435	0.27
Dodecanoic acid	30.867	0.36
Spathulenol	31.147	0.33
Hexadecanoic acid	37.773	0.90
Palmitic acid	38.792	8.78
Furo[2,3-b]quinolin-4(9H)-one	40.091	1.28
4,1-herbertenolide	40.354	0.46
9-Octadecenoic acid	40.600	0.79
9,12-Octadecadienoic acid	41.527	1.70
(1RS,2SR)-2-methyl-2'-methylpen-1-carbaldehyde	42.116	2.01
trans-Geranylgeraniol	43.587	0.73
Tetracosane	46.093	0.65
Geranyl-linalol	46.242	0.78
Octadecane	49.137	0.28
Ent-beyer-15-en-18-ol	57.383	1.25

Anti-Trichomonase vaginalis activity

The effect of *H. scabrum* methanol extract on metronidazole susceptible and resistant *T. vaginalis* strains is shown in Table 2. The minimum inhibitory concentration (MIC) was found to be 2.5 mg/mL at the end of 2 hours, 1.25 mg/mL at the end of 4 hours, and 0.6

mg/mL at the end of 24 hours after resistant *T. vaginalis* strains exposed to *H. scabrum* methanol extract. When susceptible strains are to investigated, at the end of 2 hours the MIC value of 2.5 mg/mL is the lowest dose to the study, at 4 hours later even at the concentration of 0.15 mg/mL, no live parasite is found (Table 2).

Table 2. The Viable Counts of *T. Vaginalis* Isolates Exposed to Different Concentrations of Metronidazole at the End of 2, 4 and 24 Hours

TV strains		Metronidazol Concentration								
		400 μ M	200 μ M	100 μ M	50 μ M	25 μ M	12.5 μ M	0.6 μ M	0.3 μ M	Control
N1	2 h	14.10 ³	14.10 ³	14.10 ³	15.10 ³	17.10 ³	18.10 ³	18.10 ³	18.10 ³	18.10 ³
N2		0	0	0	0	0	6.10 ³	8.10 ³	12.10 ³	17.10 ³
N1	4 h	14.10 ³	14.10 ³	14.10 ³	15.10 ³	17.10 ³	18.10 ³	18.10 ³	18.10 ³	18.10 ³
N2		0	0	0	0	0	0	6.10 ³	10.10 ³	18.10 ³
N1	24 h	14.10 ³	16.10 ³	16.10 ³	18.10 ³	18.10 ³	20.10 ³	20.10 ³	20.10 ³	20.10 ³
N2		0	0	0	0	0	0	0	12.10 ³	22.10 ³
TV strains		<i>H. scabrum</i> methanol extract concentration								
		5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.6 mg/mL	0.3 mg/mL		0.15 mg/mL		Control
N1	2 h	0	0	2.10 ³	4.10 ³	10.10 ³		10.10 ³		18.10 ³
N2		0	0	1.10 ³	2.10 ³	5.10 ³		12.10 ³		17.10 ³
N1	4 h	0	0	0	2.10 ³	3.10 ³		7.10 ³		18.10 ³
N2		0	0	0	0	0		0		18.10 ³
N1	24 h	0	0	0	0	2.10 ³		7.10 ³		20.10 ³
N2		0	0	0	0	0		0		22.10 ³

TV; *Trichomonas vaginalis*, N1; *T. vaginalis* ATCC50143 Resistance to Metronidazol, N2; *T. vaginalis* ATCC50148 Sensitive to Metronidazol.

DISCUSSION

The *T. vaginalis* is a sexually transmissible protozoan parasite and common all over the world and is found in every continent and climate and It is the commonest curable sexually transmitted infection. The prevalence of

infection varies according to the way of living and socio-cultural structure of the society. The frequency of *T. vaginalis* infection in men is not well defined, because it is usually asymptomatic and the source of continuous infection are asymptomatic men (Petrin et al., 1998:300-317).

In recent studies, this parasite has become more important as it has been found to increase the transmission rate of HIV (Polat et al., 2011:35-68).

In studies in Turkey, 5-10% in healthy women who went to private clinics *T. vaginalis* was detected. In addition, 13-25% of women admitted to the gynecology and obstetrics clinic and 50-70% of women working in brothels and women's prisons were detected (Culha et al., 2006:16-18).

Metronidazole-containing preparations are often used in the treatment of trichomoniasis. However, recently metronidazole has failed in some patients when not combined with another antibiotic, and in some patients the recurrence of the disease after a short time suggests two things. The first is that the disease is not caused by *T. vaginalis*. The second is that *T. vaginalis* has developed resistance to its medication like many living things. Although resistance studies are not sufficient, such studies have been encountered in recent years.

According to studies in the United States, metronidazole resistant *T. vaginalis* has been reported at rates varying between 4.3% and 9.6% (Kirkcaldy et al., 2012:939). In another study in Finland, 10 clinical isolates were tested positive for metronidazole resistance of these, 3 (30,0%) resistant strains were identified (Meri et al., 2000:763-767). In a 2018 study in Turkey, metronidazole resistance was tested using conventional and molecular methods and resistant isolates were

determined at a rate of 33.3% (Ozcelik et al., 2018:188-194).

Hypericum scabrum is widely used in alternative medicine. However, we have not found any study on the antiprotozoal effect of *Hypericum scabrum* on *T. vaginalis* in literature. In our study, the effect of *Hypericum scabrum* on metronidazole resistant *T. vaginalis* strains is of great importance.

CONCLUSION

It is important to underline the fact that this is the first report about the anti-*T. vaginalis* activity on *Hypericum scabrum* methanol extract. The obtained results suggest that the finding can be used to further bio-assay guided active compound isolation from this plant as promising resource.

Conflict of interest

The authors declare that there is no conflict of interest.

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