Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Investigation of in vitro anti-trichomoniasis effect of Helianthemum ledifolium L. (Mill.) varieties against Trichomonas vaginalis

Ayse Baldemir Kilic[a,*], Ulku Karaman[b], Sadi Yusufbeyoglu[a], Emine Burcu Yesilyurt[c], Turkan Mutlu Yar[b]

[a] *University of Health Sciences, Faculty of Gülhane Pharmacy, Department of Pharmaceutical Botany, Ankara, Türkiye*
[b] *Ordu University, Faculty of Medicine, Department of Medicinal Parasitology, Ordu, Türkiye*
[c] *Turkish Standards Institute, Management System Planning Directorate, Ankara, Türkiye*

ARTICLE INFO

Keywords:
Anti-protozoan
Anti-*Trichomonas vaginalis* activity
Helianthemum ledifolium varieties
Trichomonosis

Received: Nov 02, 2023
Accepted: Dec 13, 2023
Available Online: 27.12.2023

DOI:
10.5455/annalsmedres.2023.11.296

Abstract

Aim: *Trichomonas vaginalis* is a flagellated protozoan that is sexually transmitted and causes trichomonosis. It is one of the public health problems caused by sexually transmitted diseases. However, for a long time it received less attention than other parasitic and sexually transmitted diseases. Recently, the parasite has been associated with increased cases of HIV, miscarriages, infertility, pelvic inflammatory diseases and cervical and prostate cancers. Given the increasing resistance to the nitroimidazole class of drugs used in its treatment, different alternatives are needed. New drugs are increasingly being derived from natural products containing a large number of active compounds. In addition, different synthetic products or derivatives from old drugs are also used as an alternative for the treatment of trichomonosis. In this study, it was investigated whether Helianthemum ledifolium variates [*H. ledifolium* (L.) Miller var. *ledifolium* (L.) Miller; H. *ledifolium* (L.) Miller var. *microcarpum* Willk.; *H. ledifolium* (L.) Miller var. *lasiacarpum* (Willk.) Bornm.] growing naturally in Turkey. Herein aqueous and methanol extracts prepared by maceration method from the above-ground parts were evaluated for their in vitro anti-trichomoniasis activity against *Trichomonas vaginalis*.

Materials and Methods: The extracts prepared from *H. ledifolium* taxa were added to the parasite grown in culture and checked at regular intervals to check for viability. Each measurement was performed twice and averaged. Descriptive statistics of the data set are expressed as mean ± standard deviation (SD). The values of lethal doses were determined using probit analysis for certain periods of time depending on specific concentrations (LD50 and LD90).

Results: Extracts prepared from *H. ledifolium* variates inhibited the growth of *T. vaginalis* in a dose-dependent manner starting from the 4th hour. LD50 values for 4th hour were 2.39 mg for *ledifolium* water extract, 6.63 mg for *ledifolium* methanol, 2.39 mg for *lasiocarpium* water, 1.99 mg for *lasiocarpium* methanol, 5.77 mg for microcarpium water, 8.76 mg for microcarpium methanol.

Conclusion: This study data showed us that Helianthemum ledifolium varieties may be a potential natural medicine that can be used in parasite treatment. However, standardization studies should be carried out on plant extracts, and in vitro studies should be supported by in vivo studies.

Introduction

Trichomoniosis is a sexually transmitted disease caused by *Trichomonas vaginalis*, more common than gonorrhea, syphilis or chlamydia. The parasite is the most common non-viral sexually transmitted disease in the world.

The highest incidence occurs in women between 16 and 35 years of age and the overall prevalence is estimated to be 8.1% for women and 1.0% for men [1,2]. The clinical picture of trichomoniosis can be confused with other diseases involving the urinary tract and reproductive system in men and women. Definitive diagnosis can only be made by visualization of the causative agent [3–6].

Symptoms of Trichomoniosis in women include itching of the vulva, yellow-green moldy discharge from the vagina

*Corresponding author:
Email address: aysebaldemir.kilic@sbu.edu.tr (Ayse Baldemir Kilic)

and abdominal pain, but not all of these symptoms may be specific to the parasite infection. In women, urine sediment, vaginal discharge and vaginal smear samples are used in the diagnosis. The diagnosis is made by direct microscopy, culture smear and staining methods and the detection of trophozoites of *T. vaginalis* [4,6,7].

As a result of the increasing prevalence of the parasite and the emergence of strains resistant to routine drugs, alternative treatment methods are needed for effective control of the disease [4] Natural products, especially plants, are an important source of metabolites with different biological properties that can be used as active substances in the treatment of diseases, and the therapeutic use of medicinal plants is the basis for the discovery and development of new active substances, since such plants contain metabolites with various biological properties [8]. Popular knowledge of medicinal plant use is a powerful tool in the search for new active principles and WHO estimates that 65% to 80% of the population in developing countries depends on traditional medicine [9].

Helianthemum Miller (Cistaceae) species are traditionally used in Turkey for diseases such as constipation and gangrene [10], and in different countries for gastrointestinal problems, anti-inflammatory, antiulcerogenic, wound healing, antiparasitic, antimicrobial, analgesic and vasodilator [11–16]. In previous scientific studies on *Helianthemum* species, it has been reported that the high biological activities of the plants are due to the polyphenolic compounds found in their structures. They are analgesic [17], cytotoxic [18], antioxidant [19,20], antimicrobial [16,19,21–24], antiprotozoal [25–27], antigiardial [13,28] and ameobicidal [27] drugs have been shown to have important pharmacological effects by scientific studies.

Considering the literature information and the traditional use of H. *ledifolium* as a medicinal plant, it was aimed to evaluate the potential biological activity of the varieties of this species against *T. vaginalis* viability. Specifically, the antiprotozoal activity of methanol and water extracts of the above-ground parts of the variates against *T. vaginalis* was evaluated.

Materials and Methods

Propagation of Trichomonas vaginalis

In this study, *T. vaginalis* trophozoites were obtained from Sivas University Faculty of Medicine, Department of Parasitology. The parasite was cultured in Cysteine-Peptone-Liver-Maltose (CPLM) medium.

Trichomonas vaginalis CPLM (Cysteine-Peptone-Liver-Maltose)

CPLM medium Liver extract mixture: 20 g Bacto liver powder was mixed with 330 ml distilled water. The mixture was kept at 50ºC for one hour and at 80ºC for 5 minutes for protein coagulation. It was then filtered through filter paper. Ringer solution was prepared by dissolving 2 ringer tablets in 1,000 ml distilled water. Liver extract and ringer solution were mixed well. To this mixture, 32 g Peptone, 1.6 g Maltose, 2.4 g L-Cytein HCl, 1.6 g Bacto agar were added. The mixture was kept in a water bath until the agar melted and filtered after the agar melted well. 0.7 ml of 0.5% methylene blue was added to the filtered mixture. The pH of the prepared medium was adjusted to 5.8-6, 5 ml each was distributed into 125x16 mm tubes and sterilized at 121ºC for 20 min. and a sample tube was incubated at 37ºC for 24 hours to control sterilization. The tubes were stored at +4º C until inoculation.

Culture

For prepare of the medium, 1ml of inactivated human serum was added under sterile conditions (human serum inactivated at 56 ºC for half an hour is stored in the deep freezer). 0.1 ml of each of the reconstituted Penicillin, Streptomycin, and Triflucan was taken and completed to 1ml with saline and 0.2 ml of each drug was added to each tube under sterile conditions and then kept in an oven at 37ºC. After two days, it was examined for growth. Samples taken from the media were centrifuged at 1,500 g for 5 minutes to wash with sterile ringer's solution. To test the viability of trophozoites, 0.4% trypan blue was used and counted on a hemocytometer slide.

Plant material

H. ledifolium variates used in this study were collected from various localities of Turkey (Table 1).

Antiparasitic activity

The plant extracts were prepared in 0.9% saline at concentrations of 32, 16, 8, 4, 2 and 1 mg/mL and 200 µl each was dispensed into sterile eppendorf tubes. The final concentrations of *T. vaginalis* were adjusted to 51x106 trophozoites/ml and 200 µl each were added and incubated at room temperature. The viability of the parasite was checked at certain hours and noted. Control cultures were made from the tubes in which no viable cells were detected and no growth was observed in any of them. Parasites without plant extract were kept in the same medium as controls.

Statistical analysis

Descriptive statistics of the data set are expressed as mean ± standard deviation (SD) in graphic. The values of lethal doses were determined using probit analysis for specific periods of time depending on specific concentrations (LD50 and LD90).

Results

The live *T. vaginalis* trophozoites on Thoma slide are given in Figure 1 and the image of the dead parasite is given in Figure 2.

Table 1. Localities of *Helianthemum ledifolium* varieties.

Species	Location
H. ledifolium var. *ledifolium*	B4: Ankara, Beytepe campus, slopes, 980 m.
H. ledifolium var. *microcarpum*	B4: Maraş, Göksun, Ahmetli village, oak trees, 1344 m.
H. *ledifolium* var. *lasiacarpum*	B1: Çatılıdere-Foça, Çoraklar road, maquis, 15 m.

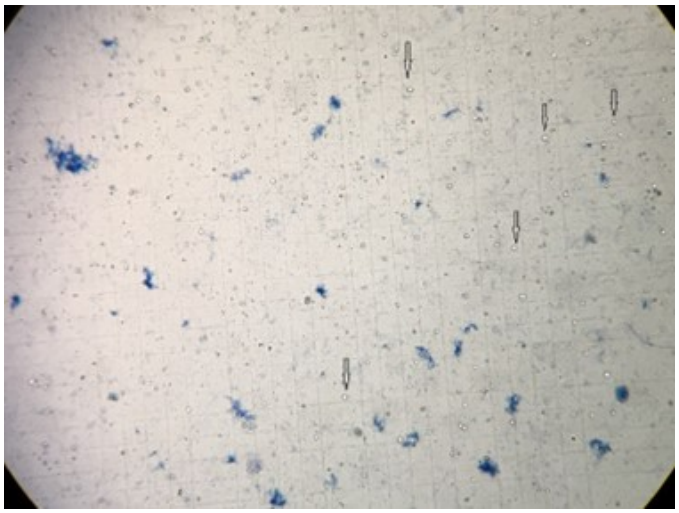

Figure 1. Live *T. vaginalis* trophozoites (0.4% trypan blue) 100X.

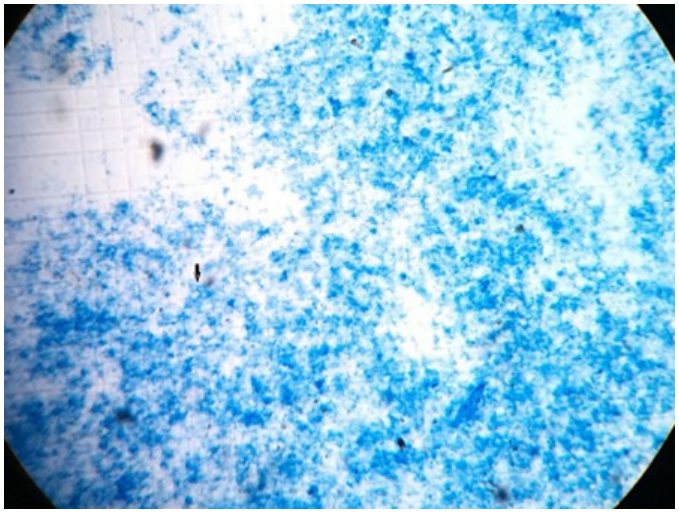

Figure 2. Disaggregated trophozoites and dead *T. vaginalis* trophozoite (0.4% trypan blue) 100X.

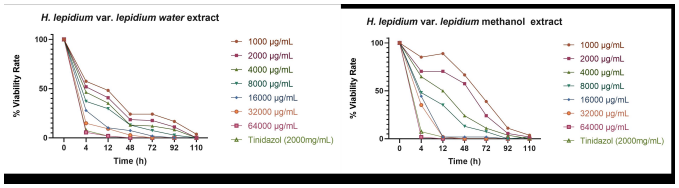

Figure 3. Percent viability of *H. ledifolium* var. *ledifolium* extracts at different concentrations against time.

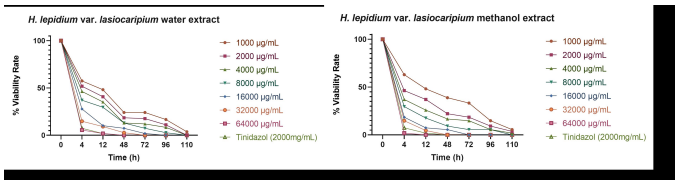

Figure 4. Percent viability rates of *H. ledifolium* var. *lasiocarpium* extracts against time at different concentrations.

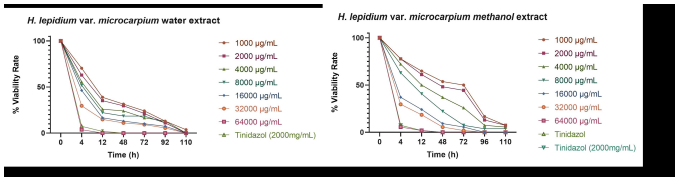

Figure 5. Percent viability rates of *H. ledifolium* var. *microcarpium* extracts against time at different concentrations.

Table 2. LD50 and LD90 values of *H. lepidium* var. l*epidium* water extract against time.

Lethal Concentration Doses		
Time	LD50 (95% confidence limit)	LD90 (95% confidence limit)
4 h	2398.807 (1651.082-3217.531) [a]	67356.542 (42267.175-131145.723)
12h	845.035 (381.726-1399.313)	34462.347 (20939.662-75380.929)
48 h	257.237 (50.976-601.655)	21442.338 (12707.758-51586.098)
72 h	146.441 (52.502-286.317)	7626.305 (5751,521-10689.314)
92 h	17.401(1.208-70.148)	2632.709 (1551.426-3951.366)
110 h	4.528(0.014-38.311)	361.764 (46.771-830.498)

-, not calculated, [a]: µg/mL.

Table 3. LD50 and LD90 values of Ledifolium methanol extract against time.

Lethal Concentration Doses		
Time	LD50 (95% confidence limit)	LD90 (95% confidence limit)
4 h	6635.454 (2568.169-15438.588)[a]	133806.301 (40138.715-6662805.192)
12h	3825.956 (3263.719-4456.022)	14348.998 (11569.98-18881.525)
48 h	1956.605 (1737.890-2179.303)	8188.163 (7122.774-9650.094)
72 h	672.188 (493.187-852.393)	4740.655 (4020.035-5758.902)
92 h	157.811 (45.991-300.592)	1198.437 (838.137- 1540.386)
110 h	6.708 (0.002-60.174)	232.168 (8.903-614.808)

-, not calculated, [a]: µg/mL.

The antiparasitic activity of *H. ledifolium* var. *ledifolium* aqueous and methanol extracts prepared at different concentrations on *T. vaginalis* trophozoites at different times are given in Tables 2, 3 and Figure 3.

At all times, there was a decrease in the viability rate as the dose increased (Figure 3). When Figure 3 is examined, it is seen that *ledifolium* aqueous extract showed a very strong antiparasitic effect after the 4th hour compared to all doses and there was a great decrease in the rate of live parasites. In addition, according to Table 2, it is seen that 50% of the trophozoites were inhibited at a concentration of 2398.807 µg/ml (LD50/4h) at 4 hours and at 110 hours it is seen that the live parasite rate approached zero. Figure 3 shows the % viability of trophozoites depending on the duration of action of *H. ledifolium* var. *ledifolium* methanol extract at different concentrations. At 110 hours, the viability rate approaches zero at all doses. *H. ledifolium* var. *ledifolium* methanol extract inhibited 50% of trophozoites at a concentration of 6635.454 µg/ml at 4 hours (Table 3). In addition, it was observed that the

Table 4. LD50 and LD90 values of *H. lepidium* var. *lasiocaripium* aqueous extract against time.

Lethal Concentration Doses		
Time	LD50 (95% confidence limit)	LD90 (95% confidence limit)
4 h	2398.809 (1864.171- 2968.334) [a]	67358.475 (47753.935- 104804.616)
12h	1228.928 (904.627-1572.829)	25296.800 (19435.243-35219.598)
48 h	156.115 (59.098- 296.222)	6599.390 (5037.189-9033.495)
72 h	296.742 (160.705-454.396)	3896.977 (3169.548-4891.553)
92 h	197.434 (84.726-336.055)	2152.643 (1688.432-2699.433)
110 h	166.860 (0.097-408.595)	588.706 (37.983-864.614)

-, not calculated, [a]: µg/mL.

Table 5. LD50 and LD90 values of *H. lepidium* var. *lasiocaripium* methanol extract against time.

Lethal Concentration Doses		
Time	LD50 (95% confidence limit)	LD90 (95% confidence limit)
4 h	1995.248 (1580.214-2431.465) [a]	34939.381 (26837.936-48473.625)
12h	1046.781 (794.474 -1309.209)	12518.383 (10214.825-16010.604)
48 h	597.498 (412.818-791 .972)	6209.500 (5156.284-7735.323)
72 h	560.148 (389.800- 732.759)	4426.290 (3725.528-5441.593)
92 h	118.446 (37.379-236.506)	1887.013 (1372.449-2459.531)
110 h	30.369 (0.851-119.725)	534.580 (149.037-931 .342)

-, not calculated, [a]: µg/mL.

Table 6. LD50 and LD90 values of *H. lepidium* var. *microcarpium* aqueous extract versus time.

Lethal Concentration Doses		
Time	LD50 (95% confidence limit)	LD90 (95% confidence limit)
4 h	5772.909 (3721.328-8564.415) [a]	149167.366 (68688.221-583718.511)
12h	562.571 (209.452-1021.649)	30632.471 (18553.713-68037.117)
48 h	323.954 (152.377-545.575)	20642.490 (14804.177-32304.573)
72 h	97.910 (24.518-229.116)	14076.424 (9814.574-23039.802)
92 h	6.305 (0.114-40.852	4216.073 (2393.820-6899.585)
110 h	0.456 (- -)	0.811 (- -)

-, not calculated, [a]: µg/mL.

Table 7. LD50 and LD90 values of *H. lepidium* var. *microcarpium* methanol extract versus time.

Lethal Concentration Doses		
Time	LD50 (95% confidence limit)	LD90 (95% confidence limit)
4 h	8767.553 (6480.386-12000.227) [a]	105525.248 (60434.371-247072.519)
12h	3409.149 (2562.198-4351.726)	52975.680 (35524.582-91884.942)
48 h	1645.998 (1340.009-1961.935)	16978.520 (13879.126-21640.339)
72 h	1257.280 (1028.426-1488.637)	8898.165 (7520.30- 10867.614)
92 h	198.024 (85.874-336.473)	2268.589 (1783.604-2848.114)
110 h	34.327 (3.347-110.577)	1041.043 (523.765-1579.164)

-, not calculated, [a]: µg/mL.

rate of live parasites approached zero at 110 hours.

The antiparasitic activity of *H. ledifolium* var. *lasiocarpium* aqueous and methanol extracts prepared at different concentrations on *T. vaginalis* trophozoites at different times are given in Tables 4,5 and Figure 4.

At all times, there was a decrease in the viability rate as the dose increased (Figure 4). When Figure 4 is examined, it is seen that *lasiocarpium* aqueous extract showed a very strong antiparasitic effect after 4 hours compared to all doses (LD50/4h: 2398.809 µg/ml) and there was a great decrease in the rate of live parasites. In addition, according to Table 4, it is seen that at the concentration of 166.860 µg/ml, the live parasite rate approached zero at the 110th hour. Figure 3 shows the % viability of trophozoites depending on the duration of action of *H. ledifolium* var. *lasiocarpium* methanol extract at different concentrations. At 110 h, the viability rate approaches zero at all doses. The methanol extract inhibited 50% of trophozoites at a concentration of 1995.248 µg/ml (LD50/4h) at 4 hours (Table 5). In addition, it was observed that the rate of live parasites approached zero at 110 hours.

The antiparasitic activity of *H. ledifolium* var. *microcarpium* aqueous and methanol extracts prepared at different concentrations on *T. vaginalis* trophozoites at different times are given in Tables 6,7 and Figure 5.

At all times, there was a decrease in the viability rate as the dose increased (Figure 5). When Figure 5 is examined, it is seen that *microcarpium* aqueous extract showed a very strong antiparasitic effect (LD50/4h: 5772.909 µg/ml) after 4 hours compared to all doses and there was a great decrease in the live parasite rate. According to Table 6, it is seen that at the concentration of 166.860 µg/ml, the live parasite rate approached zero at the 110th hour. Figure 5 shows the % viability of trophozoites depending on the duration of action of *H. ledifolium* var. *microcarpium* methanol extract at different concentrations. At 110 h, the viability rate approaches zero at all doses. The also, it was inhibited 50% of trophozoites at a concentration of 8767.553 µg/ml (LD50/4h) at 4 hours (Table 7). At the end of the experiment, it was observed that the rate of live parasites approached zero at 110 hours.

Efficacy on trophozoites increased in all extracts when exposure time and dose increased. Tinidazole 2000 ug/mL was used as standard substance. All extracts were shown to have higher efficacy than the standard substance in the range of 8000-64000ug/mL (Figure 3, 4 and 5). The difference between the mean values of the extracts with two-way anova between the application time and doses was shown by pairwise comparisons. It was shown that *lasiocarpium* methanol extracts were more active than *lasiocarpium* methanol extract, while aqueous extracts had higher activity in other extracts. As the dose and exposure time increased, the difference was observed to converge.

Discussion

Genus *Helianthemum* includes about 100 taxa in the world and some of these are important medicinal plants used in different countries for various purposes. For example, *H. lippii* [14] and *H. glomeratum* [11,25,26] have been shown to have phytomedicinal potential [29]. However, studies addressing the biological activities or phytochemistry of many of these species are not currently available in the literature. Considering some antiparasitic studies on different *Helianthemum* species in the literature; *Helianthemum*

glomeratum antiprotozoal activity has been demonstrated in vitro and in vivo, the IC50 value of *H. glomeratum* was found to be 62.92 µg/mL, in addition, the ED50 value of these extracts in a mouse model with giardiasis was found to be 0.125 mg/kg [13,30]. In another study, the *in vitro* killing potential of ethyl acetate and methanol extracts of *Helianthemum lippii* (L.) against *Acanthamoeba castellanii* cysts isolated from patients with amoebic keratitis was investigated. Both extracts proved to be potent in terms of their lethal effects on *A. castellanii* cysts and gave results comparable to chlorhexidine. Ethyl acetate was more promising in terms of cumulative lethality. It showed a fairly significant percentage of mortality over the treatment period [27].

5-Nitroimidazole derivatives (metronidazole, ornidazole and tinidazole) are used to treat *T. vaginalis*, which causes infection in the urogenital tract of both men and women. These drugs act by causing DNA damage and death of the parasite. It has been reported that the preferred metronidazole may have mutagenic and carcinogenic effects and, in this direction, alternative treatment methods have been investigated in the treatment of trichomoniasis. Süleymanoğlu et al (2021) reported that azo dyes containing uracil showed a lethal effect between 33% and 100% [31]. The researchers also determined that the dyes tested against *T. vaginalis* trophozoites showed a dose-dependent effect in vitro. The uracil-containing azo dyes used by the researchers are mostly synthetic based. . In the present study, the antiparasitic activity of water and methanol extracts of *H. ledifolium* varieties on *T. vaginalis* trophozoites was firstly investigated. The extracts prepared from *H. ledifolium* varieties inhibited the proliferation of *T. vaginalis* in a dose-dependent manner starting from the 4th hour. LD50 values for 4th hour were 2.39 mg/mL for ledifolium water extract, 6.63 mg/mL for ledifolium methanol extract, 2.39 mg/mL for lasiocarpium water extract, 1.99 mg/mL for lasiocarpium methanol extract, 5.77 mg/mL for microcarpium water extract and 8.76 mg/mL for microcarpium methanol extract. At the end of the study, high trichomonacidal activities of *H. ledifolium* extracts were observed.

Conclusion

According to the study, *H. ledifolium* taxa were found to have strong trichomonacidal activities. It is concluded that standardization studies to be carried out on extracts obtained from *H. ledifolium* varieties will may be promising in obtaining natural antiparasitic products.

Acknowledgements

The authors would like to thank Prof. Dr. Serpil Değerli, faculty member of the Department of Parasitology, Faculty of Medicine, Sivas University, for providing *Trichomonas vaginalis*.

Ethical approval

This is a study that does not require ethics committee approval.

References

1. Kissinger P. Trichomonas vaginalis: A review of epidemiologic, clinical and treatment issues. BMC Infect Dis 2015;15. https://doi.org/10.1186/s12879-015-1055-0.
2. Elizondo-Luevano JH, Verde-Star J, González-Horta A, Castro-Ríos R, Hernández-García ME, Chávez-Montes A. In vitro effect of methanolic extract of argemone mexicana against trichomonas vaginalis. Korean Journal of Parasitology 2020;58. https://doi.org/10.3347/kjp.2020.58.2.135.
3. Miller WC, Swygard H, Hobbs MM, Ford CA, Handcock MS, Morris M, et al. The prevalence of trichomoniasis in young adults in the United States. Sex Transm Dis 2005;32. https://doi.org/10.1097/01.olq.0000179874.76360.ad.
4. Radonjic I V., Dzamic AM, Mitrovic SM, Arsic Arsenijevic VS, Popadic DM, Kranjcic Zec IF. Diagnosis of Trichomonas vaginalis infection: The sensitivities and specificities of microscopy, culture and PCR assay. European Journal of Obstetrics and Gynecology and Reproductive Biology 2006;126. https://doi.org/10.1016/j.ejogrb.2005.07.033.
5. Domeika M, Zhurauskaya L, Savicheva A, Frigo N, Sokolovskiy E, Hallén A, et al. Guidelines for the laboratory diagnosis of trichomoniasis in East European countries. Journal of the European Academy of Dermatology and Venereology 2010;24. https://doi.org/10.1111/j.1468-3083.2010.03601.x.
6. Melo A, Ossa X, Fetis G, Lazo L, Bustos L, Fonseca-Salamanca F. Concordance between Clinical and Laboratory Diagnosis of Abnormal Vaginal Discharge in Chilean Women. Revista Brasileira de Ginecologia e Obstetricia 2021;43. https://doi.org/10.1055/s-0041-1735299.
7. Menezes CB, Mello M dos S, Tasca T. Comparison of permanent staining methods for the laboratory diagnosis of trichomoniasis. Rev Inst Med Trop Sao Paulo 2016;58. https://doi.org/10.1590/S1678-994620160005.
8. Patel B, Patel P, Patel R. Effect of different extracts from Celosia argentea on calcium and phosphate inhibition in vitro. Int J Pharm Pharm Sci 2011;3.
9. Sharanappa R, Vidyasagar GM. Plant profile, Phytochemistry and Pharmacology of Argemone Mexicana Linn. A review. Int J Pharm Pharm Sci 2014;6.
10. Baytop T. Türkiye'de Bitkiler ile Tedavi (geçmişte ve bugün). İstanbul Üniversitesi Yayınları 1999;3255:373.
11. Meckes M, Villarreal ML, Tortoriello J, Berlin B, Berlin EA. A microbiological evaluation of medicinal plants used by the Maya people of Southern Mexico. Phytotherapy Research 1995;9. https://doi.org/10.1002/ptr.2650090403.
12. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem 2007;104. https://doi.org/10.1016/j.foodchem.2007.01.064.
13. Barbosa E, Calzada F, Campos R. Antigiardial activity of methanolic extracts from Helianthemum glomeratum Lag. and Rubus coriifolius Focke in suckling mice CD-1. J Ethnopharmacol 2006;108. https://doi.org/10.1016/j.jep.2006.05.026.
14. Ermeli NB, Alsabri SG, Bensaber SM, Mohamed SB, Zetrini AA, Aburas KM, et al. Screening of analgesic and anti-inflammatory activities for two Libyan medicinal plants: Helianthemum lippii and Launaea residifolia. J Chem Pharm Res 2012;4.
15. Josabad Alonso-Castro A, Jose Maldonado-Miranda J, Zarate-Martinez A, Jacobo-Salcedo MDR, Fernández-Galicia C, Alejandro Figueroa-Zuñiga L, et al. Medicinal plants used in the Huasteca Potosina, México. J Ethnopharmacol 2012;143. https://doi.org/10.1016/j.jep.2012.06.035.
16. Rubio-Moraga Á, Argandoña J, Mota B, Pérez J, Verde A, Fajardo J, et al. Screening for polyphenols, antioxidant and antimicrobial activitiesof extracts from eleven Helianthemum taxa (Cistaceae) used in folk medicine in south-eastern Spain. J Ethnopharmacol 2013;148. https://doi.org/10.1016/j.jep.2013.04.028.
17. Zetrini A, Mohamed SB, Alsabri SG, Sherif FM. Helianthemum Virgatum has Analgesic-Like effect in Mice. 2009.
18. Bensaber SM, Allafe HA, Ermeli NB, Mohamed SB, Zetrini AA, Alsabri SG, et al. Chemical synthesis, molecular modelling, and evaluation of anticancer activity of some pyrazol-3-one Schiff base derivatives. Medicinal Chemistry Research 2014;23. https://doi.org/10.1007/s00044-014-1064-3.
19. Chemam Y, Benayache S, Marchioni E, Zhao M, Mosset P, Benayache F, et al. On-line screening, isolation and identification of antioxidant compounds of Helianthemum ruficomum. Molecules 2017;22. https://doi.org/10.3390/molecules22020239.

20. Mohammed FS, Kına E, Sevindik M, Doğan M, Pehlivan M. Antioxidant and antimicrobial activities of ethanol extract of Helianthemum salicifolium (Cistaceae). Indian J Nat Prod Resour 2021;12. https://doi.org/10.56042/ijnpr.v12i3.46635.
21. Rivera D, Obon C, Inocencio C, Heinrich M, Verde A, Fajardo J, et al. The ethnobotanical study of local mediterranean food plants as medicinal resources in southern Spain. Journal of Physiology and Pharmacology, vol. 56, 2005.
22. Baldemir A, Gökşen N, Ildız N, Karatoprak GŞ, Koşar M. Phytochemical Profile and Biological Activities of Helianthemum canum l. baumg. from Turkey. Chem Biodivers 2017;14. https://doi.org/10.1002/cbdv.201700052.
23. Benabdelaziz I, Marcourt L, Benkhaled M, Wolfender JL, Haba H. Antioxidant and antibacterial activities and polyphenolic constituents of Helianthemum sessiliflorum Pers. Nat Prod Res 2017;31. https://doi.org/10.1080/14786419.2016.1209669.
24. Lucia Pirvu, Ioana Nicu. Polyphenols Content, Antioxidant and Antimicrobial Activity of Ethanol Extracts from the Aerial Part of Rock Rose (Helianthemum nummularium) Species. J Agric Sci Technol A 2017;7. https://doi.org/10.17265/2161-6256/2017.01.009.
25. Meckes M, Calzada F, Tapia-Contreras A, Cedillo-Rivera R. Antiprotozoal properties of Helianthemum glomeratum. Phytotherapy Research 1999;13. https://doi.org/10.1002/(sici)1099-1573(199903)13:2<102::aid-ptr408>3.0.co;2-%23.
26. Calzada F, Alanís AD. Additional antiprotozoal flavonol glycosides of the aerial parts of Helianthemum glomeratum. Phytotherapy Research 2007;21. https://doi.org/10.1002/ptr.2031.
27. Badria FA, Hetta MH, Sarhan RM, El-Din HME. Lethal effects of Helianthemum lippii (L.) on Acanthamoeba castellanii cysts in vitro. Korean Journal of Parasitology 2014;52. https://doi.org/10.3347/kjp.2014.52.3.243.
28. Barbosa E, Calzada F, Campos R. In vivo antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. J Ethnopharmacol 2007;109. https://doi.org/10.1016/j.jep.2006.09.009.
29. Díez J, Manjón JL, Martin F. Molecular phylogeny of the mycorrhizal desert truffles (Terfezia and Tirmania), host specificity and edaphic tolerance. Mycologia 2002;94. https://doi.org/10.1080/15572536.2003.11833230.
30. Lalle M. Giardiasis in the Post Genomic Era: Treatment, Drug Resistance and Novel Therapeutic Perspectives. Infect Disord Drug Targets 2012;10. https://doi.org/10.2174/187152610791591610.
31. Süleymanoğlu N, Kubaşik P, Direkel Ş. DFT Study and Antiparasitic Activity of Some Azo Dyes Containing Uracil. J Chem 2021;2021. https://doi.org/10.1155/2021/6714151.