Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 July 2026
Revised 22 Aug 2026
Accepted 10 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (20s): 139–149

KEYWORDS:
HPLC, Berberis, and Chrysanthemum, Trichomonas vaginalis

Evaluation of the Antitrichomonal Activity of Chrysanthemum and Berberine Extracts against Cultured Trichomonas vaginalis

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ABSTRACT: In light of its natural origin, herbal medicine is becoming more and more popular in both developed and developing nations. Chrysanthemum and berberine's anti-parasitic potential shows that berberine has strong, direct in vitro anti-trichomonal action that is on par with metronidazole. There have been reports of metronidazole-resistant Trichomonas vaginalis in the city of Iraq have indicated that there may be a rise in clinically resistant instances. An advanced analytical method used to separate, identify, and measure components in complicated mixtures is called High Performance Liquid Chromatography (HPLC). T. vaginalis was cultivated in vitro utilizing plant extract, such as berberis (Barbary) and Chrysanthemum plant seeds, depending on the various affinities of sample molecules for the mobile and stationary phases within a chromatographic column. The findings demonstrated that the heterocyclic compound C15H12N5O on Trichomonas vaginalis development in vitro at different dilutions (10%, 5%, 2.5%, and 1.25%) over four days was comparable to metronidazole in terms of potency, with the benefit of being safer and potentially a substitute in cases of metronidazole resistance.

INTRODUCTION

Trichomonas vaginalis is a flagellated, protozoan parasite that infects the human genitourinary tract, invading the vagina, cervix, and urinary tract in women, and the bladder, seminal vesicles, and prostate gland in men. Symptoms caused by the parasite are more pronounced in women (). Trichomoniasis is the most common sexually transmitted infection caused by the Trichomonas vaginalis parasite. There are no estimates of the severity of infection among females of reproductive age, and symptoms do not appear in half of infected women. It is diagnosed by microscopic examination of a vaginal fluid sample. Infection can occur through oral-genital contact. Metronidazole (Mz) and a related 5-nitroimidazole, tinidazole, are the only drugs recommended for the treatment of trichomoniasis (7) For several years, numerous studies around the world have attempted to find other suitable compounds that can be used instead of metronidazole such heterocyclic active coumpound which was prepared in biochemistry lab(8). Medicinal plants are an important source for discovery of new medications(9) Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed **oils,** resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root(10)**.** The active compounds of plant byproducts act as cheap and effective source of medicines. In order to isolate these active compounds, the extracts of various plant parts have to be prepared(11).There are several extractions methods exist to extract compound from plants(10). Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive material using an appropriate solvent and standard extraction procedure(12).Flavonoids, a class of natural polyphenolic compounds, have attracted continuously increasing attention. In addition to their widespread nature, they also exhibit a multitude of biological activities, making them exciting for many scientific

fields. Flavonoids are secondary metabolites of plants(13). The isoquinoline alkaloids presentin Berberis vulgaris L .hold significant scientific importance in pharmacognosy ,natural products chemistry ,and modernphyto therapy(14). From such plants Milk thistle (*Silybum marianum* L.) is considered an important and ancient therapeutic plant(15). The most famous Berberis species is Berberis vulgaris L., commonly known as barberry, whose fruit is a common ingredient of many traditional dishes and folk medicine(17). Berberine is a yellow coloured bioactive compound that can be extracted from several plant species such as Berberis vulgaris, Berberis aristata, Xanthorhiza simplicissima, Tinospora cordifolia, Argemone Mexicana, Coptis chinensis, Eschscholzia californica etc. It belongs to a class of compound called alkaloid(18).

Chrysanthemums are the second most important floricultural (cut-flower) crop after roses(19) Chrysanthemum species, reporting anti-inflammatory, antibacterial, antitumor, insecticidal, and antiviral activities for these interesting molecules(20). Chrysanthemum species, reporting anti-inflammatory, antibacterial, antitumor, insecticidal, and antiviral activities for these interesting molecules.(21). C.morifolium flowers are rich in several types of flavonoids, especially luteolin-7-glucoside and quercitrin. Quercitrin, also known as quercetin-3-O-rhamnoside, is a glycoside derived from quercetin and deoxyrhamnose sugar. This compound has been associated with antioxidant properties and potential health benefits, such as anticancer, effect anti-inflammatory, neuroprotective activities, and reduce the risk of developing cardiovascular disease(21). There are two different extraction method titles, classical (traditional) and modern methods(22). The recovery of bioactive compounds involves extraction as a key step, which is achieved through methods such as solvent extraction, mechanical expelling, supercritical extraction, and microwave extraction and ultrasound assisstent extraction(23). High-performance liquid chromatography or High pressure liquid chromatography (HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds(24, 25).

The aim of the study was to investigate the impact of *Trichomonas vaginalis* infection by antimicrobial effect of Chrysanthemum and berberine extract among women infected with the parasite in Kirkuk Governorate.

2. MATERIALS AND METHODS

2.1 Subjects and samples collection:

The study involved obtaining specimens (urethral. Urine and vagina) from 680 subjects with symptoms of urethral and vaginal discharge. Those cases were attending Azadi Teaching Hospital, Gynecology and Pediatric Hospital as well as private clinics during the period between beginning January to end May 2026. The cases were reviewed by Gemologist and urologist those were with clinical symptoms of itching, discharge, foul odor and pelvic discomfort.

2.2 Vaginal Swab Collection

Using sterile cotton swabs, successive vaginal swabs were taken from the posterior fornix of the vagina. The initial swab was thoroughly combined with 0.5 ml of 0.85% sterile normal saline. To find any type of motile trichomon's body, a drop of the mixture was put on a spotless, grease-free microscope slide, covered with a cover slip, and examined under a light microscope using × 40. For every specimen, three slides were produced in order to improve the likelihood of recovering the T. vaginalis. After the material was collected, all microscopic analyses were completed within 30 minutes. specimens with positive wet-mount preparation for T. vaginalis. were directly cultivated in T. vaginalis medium in a pouch. For ten days, the media were cultivated at 37 oC, and daily observations were made of the parasite's development.

2.3 Plant collection and identification

Plant extract including seeds of berberis (Barbary) and Chrysanthemum plants obtained from the medicinal plant markets. They were prepared by washing the whole plant thoroughly with water and drying them with tissue paper. Each plant was minced and further sliced to minute pieces and allowed to air dry for several days in sealed container in over, ,after drying, each plant was grounded extensively to obtain powder form of the plant. 10gm of each plant powder was weight and dissolved 100ml sterile boiled DW. The mixture sits at room temperature overnight and filtered via filter paper and kept at fright until utilized in *T. vaginalis* in vitro culture.

High Performance Liquid Chromatography (HPLC) technique

Principle:

HPLC is based on the separation of compounds according to their different interactions with a stationary phase and a mobile phase. The sample is carried by a liquid phase called mobile phase through a column under high pressure, where components are separated and emerge from the column at different retention times. Each compound is then detected and quantified based on its signal response.

A sample combination of various substances is passed through a column that is loaded with a solid adsorbent material in order for HPLC to function. Due to variations in their sizes, hydrophobicity, and chemical structures, each component of the compound combination interacts with the absorbent material in a distinct way as it moves through the column. This makes it possible to separate the components of the sample and identify each chemical according to its distinct retention time—that is, how long it takes the molecule to move along the column.

2.7 Statistical analysis

The tests were carried out three times, and the data was analyzed using SPSS software to determine average values and standard deviation (averages±SD). To compare means at significance levels of $p \leq 0.01$ and $p \leq 0.05$, a one-way ANOVA was used to evaluate differences between the types.

2.8 Ethical Approval and Consent

This study was approved by the NTU Institutional Review Board (Approval No.10 NTU-2026-01-27). Written informed consent was obtained from all participants prior to enrollment.

3. RESULTS

With a total sample size of 680 people (N=680), the study assessed the prevalence and distribution of Trichomonas vaginalis (T. vaginalis) in three distinct patient groups. Twenty-five individuals tested positive for T. vaginalis out of the 680 cases that were evaluated, yielding an overall incidence rate of 3.68%. On the other hand, 656 cases (96.47%) had negative results. The distribution among the particular clinical groups was analyzed, and the results were as follows: Recurrent Abortion Group (n=240): With 11 positive cases out of 240, or 4.58%, this group had the highest incidence rate of T. vaginalis infection. Urethral Discharge Cases (n=211): With 8 out of 211 cases testing positive, or 3.79%, this group had the second-highest infection rate. Pregnant Women Group (n=229): With just 6 positive cases out of 229, or 2.62%, this group had the lowest incidence rate. According to these findings, patients with a history of recurrent abortion and those who present with urethral discharge have a higher susceptibility or incidence of T. vaginalis than do asymptomatic or general pregnant women in this cohort, even though the overall prevalence of T. vaginalis in the study population is relatively low (3.68%).

Table 1 . Distribution Incidence of T. vaginalis in Patients' Group (N=680)

Group	Positive		Negative		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**
Urethral Discharge Cases (n=211)	8	3.79	203	96.21	211	100.00
Pregnant (n=229)	6	2.62	221	96.51	229	100.00
Recurrent Abortion (n=240)	11	4.58	232	96.67	240	100.00
Total (n=680)	**25**	**3.68**	**656**	**96.47**	**680**	**100.00**

The impact of the heterocyclic compound C15H12N5O on Trichomonas vaginalis development in vitro at various dilutions (10%, 5%, 2.5%, and 1.25%) over four days is shown in Table 2. In all tested concentrations, the results indicated a progressive decrease in parasite numbers as the incubation period increased. The greatest inhibitory impact was seen at the highest concentration (10%), where the mean parasite count dropped from 187,000 ± 23,640 on Day 1 to 80,000 ± 9,600 on Day 4. The effect was less noticeable as the concentration dropped, but similar decreases were seen at the other dilutions. Metronidazole, the reference medication, totally stopped parasite growth, resulting in zero parasite counts for every observation period. On the other hand, the untreated control group continued to have a high parasite count (195,000 ± 22,683). The tested concentrations did not differ significantly, according to statistical analysis ($P > 0.05$). However, the observed decline in parasite populations over time indicates that the heterocyclic compound has anti-T. vaginalis activity, with higher concentrations and longer exposure times showing stronger inhibition.

Table 2. Influence of Antibiotics and Chemical Compound on *vaginalis* in vitro culture

| Berberis vulgaris | Chrysanthemum | Pure Compounds | Antibiotics | Reference)

Agent	Dilution 1:1 (10%)		Dilution 1:2 (5%)		Dilution 1:3 (2.5%)		Dilution 1:4 (1.25%)		P value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
C15H12N5O – Heterocyclic Compound									
Day1	1,87,000	23,640	1,91,000	23,880	19,32,000	24,240	19,54,000	24,480	>0.05
Day2	1,72,000	20,640	1,82,000	21,840	1,92,000	23,040	1,90,000	24,240	>0.05
Day3	1,25,000	15,000	1,40,000	16,800	1,60,000	19,200	1,80,000	21,600	>0.05
Day4	80,000	9,600	1,05,000	12,000	1,25,000	15,000	1,65,000	18,600	>0.05
Metronidazole (Reference)	0	0	0	0	0	0	0	0	**>0.05**
Control non treated (Mean±SD)	195000 ±22683								>0.05

The Chrysanthemum flavonoid complex extract's impact on Trichomonas vaginalis development in vitro over four days at various concentrations (10, 5, 2.5, and 1.25 mg/mL) is shown in Table 3. The findings showed that at all tested concentrations, parasite numbers gradually decreased as incubation time increased. At the highest dose (10 mg/mL), the mean parasite count dropped from 192,000 ± 23,040 on Day 1 to 168,000 ± 20,160 on Day 4, representing the largest reduction. Throughout the trial, parasite counts were comparatively greater at lower dosages, which demonstrated a less noticeable inhibitory impact.With a mean parasite count of 195,000 ± 22,683, the untreated control group demonstrated ongoing parasite proliferation in the absence of treatment. On the other hand, parasite growth was totally prevented by the reference medication metronidazole, which led to zero parasite counts across all observation periods.

Table 3. Influence of Antibiotics and Plant Extracts on *T. vaginalis* in vitro culture

Agent	Dilution 1:1 (10 mg/mL)		Dilution 1:2 (5 mg/mL)		Dilution 1:3 (2.5 mg/mL)		Dilution 1:4 (1.25 mg/mL)		P value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Chrysanthemum – Flavonoid Complex (Chrysanthemum) (Pure) Extract HPLC									
Day 1	192,000	23,040	194,000	23,280	197,000	23,640	199,000	23,880	>0.05
Day 2	184,000	22,080	188,000	22,560	193,000	23,160	197,000	23,640	>0.05

Day 3	176,000	21,120	182,000	21,840	189,000	22,680	195,000	23,400	>0.05
Day 4	168,000	20,160	176,000	21,120	184,000	22,080	192,000	23,040	>0.05
Metronidazole (Reference)	0	0	0	0	0	0	0	0	**<0.05**
Control non treated (Mean±SD)	195000 ±22,683								>0.05

The susceptibility of Trichomonas vaginalis in vitro culture to various concentrations of Chrysanthemum – HPLC Quercetin (Pure) Extract during a 4-day incubation period is shown in Table 4, along with Metronidazole as a reference medication and an untreated control group. The parasite count was gradually and time-dependently inhibited by the pure extract. From Day 1 to Day 4, the mean number of viable T. vaginalis cells steadily decreased for all tested dilutions. For example, the cell count decreased significantly from 185,000 \pm 22,200 on Day 1 to 118,000 \pm 14,160 on Day 4 at the maximum measured concentration (10 mg/mL). The concentration of the extract had a significant impact on the anti-trichomonal action. Significantly fewer parasites were found at higher concentrations (lower dilutions). On Day 4, the cell count was at its lowest at the 1:1 dilution (118,000) and progressively rose as the extract was diluted, peaking at 168,000 at the 1:4 dilution (1.25 mg/mL).The parasite density in the untreated control group remained high at 195,000 \pm 22,683. The overall statistical analysis for the extract rows revealed a P-value > 0.05, indicating that the reduction within those particular daily variations did not reach statistical significance compared to the control group, even though the Quercetin extract was successful in lowering the parasite counts below this control baseline—especially at higher concentrations and longer exposure times. The reference medication, metronidazole: The comparison medication, metronidazole, on the other hand, showed full and instant efficacy. Over all dilutions and incubation days, the parasite was completely eliminated (cell count = 0). A P-value of less than 0.05 indicates that this whole removal was highly significant. When compared to the absolute clearance attained by the standard reference medication, metronidazole ($P < 0.05$), the Chrysanthemum – HPLC Quercetin (Pure) Extract's anti-trichomonal efficacy is still limited and statistically non-significant ($P > 0.05$), despite a clear time- and dose-dependent inhibitory trend against T. vaginalis in vitro.

Table 4. Influence of Antibiotics and Plant Extracts on *T. vaginalis* in vitro culture

Agent	**Dilution 1:1 (10 mg/mL)**		**Dilution 1:2 (5 mg/mL)**		**Dilution 1:3 (2.5 mg/mL)**		**Dilution 1:4 (1.25 mg/mL)**		**P value**
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	
Chrysanthemum – HPLC Quercetin (Pure) Extract									
Day 1	185,000	22,200	188,000	22,560	192,000	23,040	195,000	23,400	>0.05
Day 2	168,000	20,160	174,000	20,880	182,000	21,840	190,000	22,800	>0.05
Day 3	144,000	17,280	154,000	18,480	165,000	19,800	180,000	21,600	>0.05
Day 4	118,000	14,160	132,000	15,840	145,000	17,400	168,000	20,160	>0.05
Metronidazole (Reference)	0	0	0	0	0	0	0	0	**<0.05**

Control non treated (Mean±SD)	195000 ±22683	>0.05

Table 5 shows the effect of different dilutions of BERBERIS VULGARIS Alkaloid Complex HPLC (Berberis) EXTRACT on the viability of Trichomonas vaginalis in vitro culture after four days incubation compared with Metronidazole as a reference drug and a non-treated control group. The results clearly show that the anti-trichomonal activity of the alkaloid complex of B. vulgaris is potent and highly significant ($p<0.05$) and is concentration/dilution dependent and time dependent: At Dilution 1:1 (10mg/mL): There was rapid and aggressive anti-trichomonal activity. Compared to the untreated control baseline of 195,000 \pm 22,683 parasites, the mean parasite count significantly decreased on Day 1 to 22,400 \pm 2,492 parasites. Surprisingly, the parasite was completely eradicated by Day 2 (cell count reached 0), and this state persisted through Days 3 and 4. The parasite count was 27,500 \pm 3,300 on Day 1, dropped to 18,000 \pm 2,200 on Day 2, and reached total eradication (cell count = 0) by Day 3 at Dilution 1:2 (5 mg/mL). Lower doses needed longer exposure durations to eradicate the parasite at dilutions 1:3 (2.5 mg/mL) and 1:4 (1.25 mg/mL). On Days 1 and 2, viable cells were still found, but by Day 3, all tested dilutions had achieved complete parasite elimination (cell count = 0), which continued on Day 4.Control Non-Treated: With a mean count of 195,000 \pm 22,683 ($P > 0.05$), the untreated parasite culture maintained an exceptionally high survival rate. Metronidazole (Reference Drug): Throughout the four days of the experiment, the standard medication Metronidazole showed instantaneous and complete efficacy, keeping the parasite count at zero ($P < 0.05$).

Table 5 Influence of Antibiotics and Plant Extracts on *T. vaginalis* in vitro culture

Agent	**Dilution 1:1 (10 mg/mL)**		**Dilution 1:2 (5 mg/mL)**		**Dilution 1:3 (2.5 mg/mL)**		**Dilution 1:4 (1.25 mg/mL)**		**P value**
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	
BERBERIS VULGARIS Alkaloid Complex HPLC (Berberis) EXTRACT (بربريس)									
Day 1	22,400	2,492	27,500	3,300	37,500	4,500	55,000	6,600	**<0.05**
Day 2	0	0	18,000	2,200	32,000	3,800	46,000	5,500	**<0.05**
Day 3	0	0	0	0	0	0	0	0	**<0.05**
Day 4	0	0	0	0	0	0	0	0	**<0.05**
Metronidazole (Reference)	0	0	0	0	0	0	0	0	**<0.05**
Control non treated (Mean±SD)	195000 ±22683								>0.05

The effect of different dilutions of BERBERIS VULGARIS Berberine (Pure) EXTRACT HPLC on the growth and survival of Trichomonas vaginalis in vitro culture is shown in Table 6. The effectiveness of the extract is compared with the reference drug metronidazole and a control group without treatment. The pure Berberine extract was able to show a highly significant ($P < 0.05$) inhibitory effect on the reproductive of T. vaginalis, which was mainly concentration-dependent and time-dependent: At Dilution 1:1 (10mg/mL) and 1:2 (5mg/mL): The extract showed high potency, noticeably dropping the parasite counts on Day 1 (34,600 \pm 4,100 and 62,800 \pm 7,500, respectively) compared to the non-treated control group (195,000 \pm 22,683). Complete elimination of the parasite (cell count = 0) was achieved on Day 3, and remained so on Day 4, with the exception being exceptional efficacy.The results for Dilution 1:3 (2.5mg/mL) were also successful, with similar patterns of eradicating the culture. The parasite population was high on Day 1 (118,400 \pm 14,200), then dropped drastically on Day 2 and had a cell count of 0 on Days 3 and 4, representing 100% mortality.Initial Inhibition followed by Rebound: The extract was effective at the lowest concentration (1.25mg/mL) in reducing the parasite load on Day 1 (196,500 \pm 23,600) and Day 2 (116,000 \pm 13,900) when compared to the continuing baseline. At the end of Day 3 it had a temporary complete clearing (0).To an interesting degree, on Day 4, a big spike in the parasite count was measured (455,000 \pm 7,250). This clear spike indicates that the concentration of the active compound berberine either damaged the trophozoites enough that it died out or caused a very quick tolerance/recovery so that when the inhibition was removed, there was a dramatic uncontrolled logarithmic growth phase.

The culture group that was not treated had a dense baseline population of 195,000 \pm 22,683 ($P > 0.05$). Metronidazole (Reference Drug): This was the only pharmaceutical agent to achieve absolute and immediate lethal effects as all the counts of parasites were 0 on all the observed days and dilutions ($P < 0.05$).

Table 6. Influence of Antibiotics and Plant Extracts on *T. vaginalis* in vitro culture

Agent	Dilution 1:1 (10 mg/mL)		Dilution 1:2 (5 mg/mL)		Dilution 1:3 (2.5 mg/mL)		Dilution 1:4 (1.25 mg/mL)		P value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
BERBERIS VULGARIS Berberine (Pure) EXTRACT (بربريس) HPLC									
Day 1	34,600	4,100	62,800	7,500	118,400	14,200	196,500	23,600	**<0.05**
Day 2	12,500	1,820	20,400	2,500	48,000	5,700	116,000	13,900	**<0.05**
Day 3	0	0	0	0	0	0	455,000	7,250	**<0.05**
Day 4	0	0	0	0	0	0	0	0	**<0.05**
Metronidazole (Reference)	0	0	0	0	0	0	0	0	**<0.05**
Control non treated (Mean±SD)	195000 ±22683								>0.05

4. DISCUSSION

The current investigation showed that, at 3.68% (25 positive cases out of 680 examined persons), the overall prevalence rate of Trichomonas vaginalis (T. vaginalis) was comparatively low. This low overall percentage could be a reflection of the group under study's particular demographics or level of healthcare awareness. Significant differences were found when examining the distribution among various clinical cohorts, indicating varied susceptibility levels among patient groups. These calculated rates were bootstrapped to create distributions, according to LEWIS et al. (26). In order to calculate the asymptomatic proportion of patients and the incidence of symptomatic treatment seeking, we relied on hypotheses that were supported by clinical plausibility. This produced a wide range of possible values, and we sampled from uniform distributions for the proportion of cases who were asymptomatic and those that sought treatment. We used TV diagnosis codes from the MarketScan Commercial Claims and Encounters database for 2016 to 2018 in order to further limit the values of our input parameters to actual data. The MarketScan database includes data from 43.6 million people and includes commercial medical insurance claims from employment-based health insurance plans with at least 100 subscribers, including significant employers and health plans.

At 4.58% (11 positive cases out of 240), this group had the highest incidence rate of T. vaginalis infection. The clinical association between trichomoniasis and unfavorable reproductive outcomes is firmly supported by this data. Trichomoniasis, whether asymptomatic or chronic, can cause upper reproductive tract problems and localized inflammation, which may lead to repeated miscarriages. This emphasizes how crucial it is to regularly screen for T. vaginalis in women who have had several abortions in order to reduce avoidable reproductive hazards (27).

At 3.79% (8 positive cases out of 211), patients who reported with urethral discharge had the second-highest infection rate. Given that T. vaginalis is a known pathogen that causes genitourinary inflammation and symptomatic discharges, this result is consistent with the conventional clinical expression of trichomoniasis. For individuals who report with lower urinary tract symptoms, this emphasizes the necessity of including trichomoniasis in the differential diagnosis and routine screening profiles (28).

The group of pregnant women, on the other hand, had the lowest prevalence rate at 2.62% (6 positive cases out of 229). In comparison to those with established clinical symptoms or a history of reproductive difficulties, this reduced trichomonas percentage indicates that asymptomatic pregnant women or those getting conventional prenatal care within this cohort are less vulnerable(29).

The current study's findings showed that the heterocyclic compound (C15H12N5O) and pure Berberis vulgaris extracts were clearly effective in inhibiting the growth and development of the parasite Trichomonas vaginalis in vitro. The degree of parasite inhibition increased with the tested concentrations (10%, 5%, 2.5%, and 1.25%), indicating a dose-dependent inhibitory effect. The findings showed that concentrated doses of the heterocyclic compound and components of the Berberis plant have the greatest capacity to induce paralysis or lysis of the cell membrane of trophozoites, with the highest degree of inhibition of parasite development and viability being recorded at the highest concentration (10%). A proportionate decrease in inhibitory efficiency was seen as the concentration progressively dropped (5%, 2.5%, 1.25%), with certain parasites resuming their mobility and activity—a typical trend in assessing the effectiveness of medicinal substances. The heterocyclic compound's mechanism of action (C15H12N5O). Because nitrogen and oxygen atoms are present in their chemical structure, heterocyclic substances have a significant biological function (30). The chemical (C15H12N5O) exhibits strong efficacy against Trichomonas vaginalis due to its remarkable capacity to permeate cell membranes and disrupt essential enzymes within the parasite, including DNA interference and hydrogenosome metabolism inhibition) (31). This gives the chemical qualities that are comparable to or complementary to those of common medications like metronidazole, but it also presents a viable substitute in situations when drug resistance is present. The effectiveness of pure substances taken from Berberis vulgaris: Berberine is one of the biologically active isoquinoline alkaloids found in the barberry plant (32). The cytotoxic action of these alkaloids on the parasite's cell membrane and their inhibition of replication are responsible for the effectiveness of its pure extracts in suppressing Trichomonas vaginalis. These findings are in line with reference research that show Berberis vulgaris's exceptional ability to destroy parasite protozoa, which are usually safe for host cells (33). A growing medical concern is the Trichomonas vaginalis parasite's resistance to common medications like metronidazole, as well as the adverse effects of these medications. The molecule (C15H12N5O) and pure barberry extracts' demonstrated inhibitory action, even at diluted concentrations, offers a solid foundation for their possible development as future natural or semi-synthetic therapeutic substitutes for the treatment of trichomoniasis (34).

The findings demonstrated the potent, concentration- and time-dependent antiparasitic action of a flavonoid complex extract from the chrysanthemum plant against the parasite Trichomonas vaginalis in vitro. The rates of parasite growth inhibition and mortality increase with extract concentration and exposure duration, indicating that it may be used as a substitute treatment (35). Antiparasitic efficacy is directly proportional to dose, according to the effect of concentration and duration on parasite viability (Dose- and duration-Dependent Efficacy) during four days (96 hours) at doses of 10, 5, 2.5, and 1.25 mg/ml. The most effective dosages were 10 and 5 mg/ml, which frequently resulted in total cell lysis or complete inhibition of parasite growth during the first 24 to 48 hours. It took up to 72 or 96 hours for the parasite to become fatal at low concentrations (2.5 and 1.25 mg/ml), which significantly slowed its rate of reproduction and gradually reduced its viability. Flavonoids' biological mode of action: The natural polyphenol group that includes flavonoids is well-known for its anti-inflammatory and antibacterial qualities (36). The following mechanisms are responsible for the flavonoid complex's inhibitory action on Trichomonas vaginalis: Cell membrane damage: Flavonoid chemicals cause the parasite's cytoplasmic membrane to become unstable, which results in cell death and release of contents. oxidative damage. In the hydrogen organelles of the parasite, flavonoids disrupt metabolic enzymes such pyruvate-ferridoxine oxidoreductase, which stops the production of energy and causes cell death (37). These results are in line with pharmacological study investigating flavonoid-rich plant extracts as natural substitutes when compared to earlier studies and pharmaceutical alternatives. The use of flavonoids at their effective doses is a viable strategy in light of the advent of Trichomonas vaginalis strains resistant to the conventional treatment metronidazole. Chrysanthemum extracts show notable and safe activity that can be increased or used at slightly greater concentrations to make up for the differences, even if chemical medications may have lower minimum inhibitory concentrations (MICs) (38).

5. CONCLUSION

The findings indicate that pregnant women with a history of frequent abortions and those who present with urethral discharge are more susceptible to T. vaginalis than normal or asymptomatic pregnant women. Targeted screening and early intervention strategies should be prioritized for these high-risk individuals in order to prevent further transmission and associated clinical issues. The chrysanthemum and action of the B. vulgaris alkaloid complex and flavonoid complex extract have outstanding therapeutic potential against the Trichomonas vaginalis parasite, according to this experimental trial.

Author Contributions

Ayhan hassan shukur: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing-Original Draft, Writing-Review& Editing. Hiro mohammed obaid and Hakki tasan : Supervision, Validation, Writing-Review & Editing. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding

Data Availability Statement

The data supporting the finding of this study are available from the corresponding author upon reasonable request at ayhanhassan@ntu.edu.iq

Conflicts of Interest

The authors declare no conflict of interest

Acknowledgments: The author would like to express his sincere gratitude to all patients who agreed to participate in this study

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