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Antimicrobial Potential of Phenolic Compounds from Seidlitzia Rosmarinus and Ziziphus Spina-Christi against Trichomonas Vaginalis: A Natural Alternative to Conventional Therapy

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Abstract: This study investigates the presence of Trichomonas vaginalis among women visiting Samarra General Hospital and private clinics. The parasite was successfully cultured in TYM medium, with the logarithmic growth phase observed at 48 hours. Phenolic compounds were extracted from Seidlitzia rosmarinus and Ziziphus spina-christi leaves and identified using High-Performance Liquid Chromatography (HPLC). Results showed that phenolic extracts from both plants exhibited significant inhibitory effects on parasite growth, with varying efficacy based on concentration and exposure time. Kaempferol acid and Caffeic acid were identified as key phenolic compounds, contributing to the antimicrobial, antioxidant, and anti-inflammatory properties observed. These findings highlight the potential of plant-derived phenolic compounds as alternative treatments for Trichomonas vaginalis infections, offering a promising avenue for further research.

Key points: Trichomonas vaginalis, phenolic compounds, Seidlitzia rosmarinus, Ziziphus spina-christi, HPLC, antimicrobial activity, natural treatment, parasitic infection.

****Introduction****

Trichomoniasis is a sexually transmitted disease caused by a type of anaerobic parasite known as *Trichomonas vaginalis* (1).

The *Trichomonas vaginalis* parasite is classified as an atrial-flagellate that inhabits the external cavities and vestibules of humans. It resides within the genitourinary tracts of both sexes, causing trichomoniasis in females, which is characterized by vaginal discharge. In males, the infection is often asymptomatic or may lead to urethritis (2). This parasitic infection is widespread globally, particularly in developed countries, as it is considered a sexually transmitted disease (3).

Plants are a rich source of active compounds used in the pharmaceutical industry. These compounds may either naturally exist in plants or be secondary metabolites produced through metabolic processes (4).

Research Objectives

1. To investigate the presence of *Trichomonas vaginalis* among women visiting Samarra General Hospital and select private clinics, cultivate the parasite, and determine its logarithmic growth phase.
2. To extract phenolic compounds from the plants *Seidlitzia rosmarinus* (al-shanan) and *Ziziphus spina-christi* (sidr leaves), and identify them using High-Performance Liquid Chromatography (HPLC).

3. To study the effect of these compounds on the in vitro growth of the parasite and compare their efficacy with the drug Metronidazole (Flagyl).

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Materials and Methods

Sample Collection

Samples were collected from November 1, 2012, to June 1, 2013, totaling 320 samples from women visiting the outpatient department at Samarra General Hospital and private clinics.

2.1 Wet Swab Test

The test followed the method described in (4). A sterile cotton swab and a medical speculum were used by a specialist physician. Two milliliters of normal saline were added to the sample, mixed thoroughly, and a drop was placed on a glass slide for examination under a light microscope at magnifications of ×10 and ×40. The parasite was identified based on its jerky movement, flagellar motion, and undulating membrane among epithelial cells.

2.2 Culture Medium

Samples were cultured using Diamond's TYM medium, consisting of trypticase-yeast extract maltose, prepared under sterile conditions as described in (5).

2.3 Plants Used in the Study

- *Seidlitzia rosmarinus* ✓
- *Ziziphus spina-christi* ✓

2.4 Extraction and Identification of Phenolic Compounds

Phenolic compounds were extracted from plant samples following the method describe (6) and identified using High-Performance Liquid Chromatography (HPLC) according to(7).

2.5 Parasite Cultivation and Determination of the Logarithmic Growth Phase

The logarithmic growth phase of *Trichomonas vaginalis* cultivated in TYM medium was determined by counting the parasite every 24 hours over three days using a hemocytometer.

2.6 Effect of Extracted Phenolic Compounds on Parasite Growth

Three concentrations of phenolic compounds extracted from *Seidlitzia rosmarinus* and *Ziziphus spina-christi* leaves (25, 50, and 100 mg/mL) were prepared and added to the culture medium, resulting in final concentrations of 1, 0.5, and 0.25 g/mL. Each concentration was tested in triplicate.

Results and Discussion

The parasite *Trichomonas vaginalis* was successfully cultivated in TYM medium, reaching a growth rate of 10.08×10510.08 \times 10^510.08×105 cells in the logarithmic phase at 48 hours (Figure 1). The effect of phenolic compounds extracted from the studied plants was subsequently examined.

The results indicated that phenolic extracts from *Seidlitzia rosmarinus* exhibited inhibitory effects on parasite growth at concentrations of 100 and 50 mg/mL within 24 hours, and at a concentration of 25 mg/mL within 48 hours (Figure 2). Similarly, phenolic extracts from *Ziziphus spina-christi* leaves inhibited parasite growth at 100 mg/mL within 24 hours, at 50 mg/mL within 48 hours, and at 25 mg/mL within 72 hours (Figure 3).

The inhibitory effects of these extracts can be attributed to the presence of phenolic compounds with antimicrobial properties. The efficacy of the phenolic extracts may be due to the presence of *Kaempferol acid*, which has demonstrated antimicrobial activity (8), as well as antioxidant and anti-inflammatory properties (9). Additionally, studies have shown that *Kaempferol acid* effectively inhibits the growth of various bacteria and fungi (10).

Other phenolic compounds, such as *Chlorogenic acid*, *Ferulic acid*, and *Getistic acid*, are also known to exhibit potent antibacterial and anti-inflammatory effects (11).

3.2 Identification of Phenolic Compounds in Plants

The results of High-Performance Liquid Chromatography (HPLC) analysis revealed that *Seidlitzia rosmarinus* contains phenolic compounds, including *Gallic acid*, *Kaempferol*, *Vanillic acid*, *Ferulic acid*, *Ellagic acid*, *Getistic acid*, and *Chlorogenic acid*. The retention times of these compounds matched those of standard phenolic compounds, as shown in Table 1.

Table 1. Phenolic Compounds Identified in *Seidlitzia rosmarinus*

Phenolic Compound	Standard Retention Time (min)	Extracted Retention Time (min)	Concentration (mg/g)
Gallic acid	0.95	1.12	6.35
Kaempferol	1.74	1.79	27.01
Vanillic acid	2.72	2.79	9.80
Ferulic acid	4.11	3.93	12.47
Ellagic acid	4.96	5.45	11.80
Getistic acid	6.07	5.89	5.89
Chlorogenic acid	6.79	6.84	26.12

Identification of Phenolic Compounds in *Ziziphus spina-christi*

The results of High-Performance Liquid Chromatography (HPLC) analysis also confirmed that the leaves of *Ziziphus spina-christi* contained phenolic compounds, including *Hydroxyl benzoic acid*, *Syringic acid*, *Caffeic acid*, *Chlorogenic acid*, *Coumaric acid*, *Catcatchen acid*, *Ferulic acid*, and *Apigenin-7-glycoside*. The retention times of these compounds matched those of the standard phenolic compounds, as shown in Table 2.

The highest concentration was observed for *Caffeic acid*, followed by *Hydroxyl benzoic acid* and *Syringic acid*. Notably, *Caffeic acid* has diverse and significant pharmacological properties, including inhibitory effects on the spread of cancer cells (12).

Table 2. Phenolic Compounds Identified in *Ziziphus spina-christi* Leaves

Phenolic Compound	Standard Retention Time (min)	Extracted Retention Time (min)	Concentration (mg/g)
Hydroxyl benzoic acid	1.14	1.14	19.10
Syringic acid	1.81	1.82	15.06
Caffeic acid	2.65	2.64	30.43
Chlorogenic acid	3.55	3.52	8.89
Coumaric acid	4.46	4.43	11.44
Ferulic acid	5.30	5.08	10.66
Catcatchen acid	6.08	6.03	2.00

Apigenin-7-glycoside	6.81	6.81	0.75

Conclusion

This study demonstrated that phenolic compounds extracted from *Seidlitzia rosmarinus* and *Ziziphus spina-christi* possess significant inhibitory effects on the growth of *Trichomonas vaginalis*, with variations in efficacy depending on concentration and exposure time. The findings highlight the potential of these natural compounds, particularly *Caffeic acid* and *Kaempferol acid*, as promising alternatives to conventional treatments due to their antimicrobial and antiparasitic properties.

Recommendations

1. Conduct further studies to elucidate the precise mechanisms of action of these phenolic compounds against the parasite.
2. Expand research to assess the clinical efficacy of these compounds in treating infected patients.
3. Explore the potential of combining plant extracts with conventional drugs to enhance effectiveness and reduce side effects.
4. Investigate the impact of these phenolic compounds on other parasites and microorganisms to broaden their therapeutic applications.
5. Promote research on utilizing local plants as sources of bioactive compounds for developing cost-effective and sustainable treatments.

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