Investigation of *Tagetes erecta* L. extract as a natural solution to vaginitis problems

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ABSTRACT

One of the most common health problems experienced by women is vaginal discharge that occurs for various reasons. Vaginitis, which manifests with such discharge, is an important disorder seen in women. This study aimed to investigate the effect of extracts obtained from *Tagetes erecta* on some microorganisms that cause discharge outside the menstrual cycle in women, to produce gels and pads that can prevent this discharge and raise awareness of women about this issue. *T. erecta* is an important ornamental plant grown in parks and gardens. The *T. erecta* samples used as material in the study were collected and dried. Three different extracts were obtained from the plant's flowers, leaf + stem, and flower + leaf + stem. The antimicrobial activities of these extracts were investigated on *Enterococcus faecalis, Escherichia coli* and *Lactobacillus acidophilus* bacteria and *Candida albicans*, which cause vaginitis and urinary tract infections. It was determined that the flower and flower + leaf + stem extracts were effective in bacteria but not in *C. albicans*. The flower extract was also used in the production of gels and pads considering that it did not show much effect on *L. acidophilus*, which is important for the vaginal flora. In the study, short interviews were also undertaken with women to elicit their knowledge about vaginal discharge. As a result of the study, using the flower extract with a higher antimicrobial effect as compared with the other extracts, we produced natural, practical, useful and easily accessible gels and pads that can be used against vaginal discharge as an alternative to synthetic drugs that affect other organs. These products can also help treat urinary tract infections. However, the present study showed the importance of raising women's awareness about this condition as one of the priorities.

**Key words:** Gel, pad, *T. erecta*, urinary tract infections, vaginal discharge, vaginitis.

INTRODUCTION

Vaginal discharge is one of the most common complaints of women throughout their lives. Vaginitis, generally manifesting with such discharge, constitutes the most frequent disorder seen in women. Vaginitis is a disease that presents with malodorous discharge, itching, and pain due to the inflammation of the external genital organ and vaginal area. This disease can be caused by fungal, bacterial and protozoal infections, irritant substances, drugs used, tumors and hormonal changes. In addition, vaginitis can be transmitted from public toilets, as well as poor choice of underwear, use of vaginal cleaning soap, not keeping the vagina clean, types of pads used, and sexual abuse (Balci and Çapar, 2005; Kaygusuz et al., 2014). A wide variety of vaginitis is seen depending on the disease factor, with the most common being detailed as follows:

- **Bacterial vaginitis:** Bacterial infection is the most common cause of vaginitis and presents with a discharge described to have fishy smell. There is a vaginal flora inside the vagina (pH = 3.8-4.5), in which all microorganisms that do not harm people are present. Microorganisms living in the vaginal flora do not harm women since they live in...
certain numbers and maintain a balance. However, the changes in the normal vaginal flora lead to the loss of hydrogen peroxide producing lactobacilli and emergence and dominance of anaerobic gram-negative bacteria (Prevotella, Porphyromonas and Bakteroides) and other bacteria, including Gardnerella vaginalis, Neisseria gonorrhoeae, Enterococcus, and Streptococcus. Among these, G. vaginalis is the most common while N. gonorrhoeae is mostly seen during childhood and postmenopausal period and it is usually asymptomatic.

-Fungal vaginitis: This is caused by Candida albicans and manifests with severe itching and a white, cottage cheese-like discharge. This disorder is experienced by 75% of adult women at least once in their lives.

-Trichomonas vaginalis vaginitis: This has a high rate of transmission, sexually transmitted, and seen in both males and females. The parasite that causes this condition (T. vaginalis) is a flagellated protozoan.

-Desquamative inflammatory vaginitis: This condition causes bleeding in the form of epithelial cell shedding, abundant discharge, burning in the vagina, and occasional spotting. It occurs with the increase of streptococci and has no effective treatment.

There are many different types of vaginitis caused by estrogen deficiency and improper use of vaginal products, such as tampons, cream, and soap (Kaygusuz et al., 2014). The treatment of these conditions is critical because if left untreated, they can lead to significant health risks such as pelvic inflammatory disease, infertility, chronic pelvic pain, ectopic pregnancy, and uterine, cervical and ovarian cancer (Breshears et al., 2015). These conditions are tried to be treated with vaginal tablets, creams, antibiotics, and antiparasitics but some of these drugs are not effective (Balci and Çapar, 2005; Kaygusuz et al., 2014). One of the most important points in terms of health is that most of these drugs are produced synthetically. These synthetic substances in the body damage organs, especially the liver and kidneys. Therefore, using natural ingredients as an alternative to synthetics; that is, developing traditional medicine methods is important for health.

Traditional medicine is the oldest method, in which various parts of plants are used to treat diseases and infections. Plants used in these methods are called medicinal plants, and they have active substances (bioactive components = secondary metabolites) utilized in the treatment of many diseases (Sumathi et al., 2010). These substances can be listed as peptides, unsaturated long-chain aldehydes, alkoidal components, some essential oils, phenols, ethanol, chloroform, methanol, and compounds soluble in butanol (Seyednejad et al., 2009). Since these bioactive compounds are widely used as an alternative source in modern medicine (Van Wyk and Wink, 2009), there is an increasing number of studies investigating medicinal herbs.

One of these plants, Tagetes erecta, belongs to the Asteraceae (Compositae) family and originates from North-South America (Motamedi et al., 2015). In addition to being an ornamental plant, it has a long history in traditional and folk medicine due to its secondary metabolites and is still used in many countries (Verma and Verma, 2012). This plant is used in food and tea in Thailand with its rich content of flavonoids and phenolic compounds (gallic acid, scopoletin, ferulic acid, quercetin) and anticarcinogenic properties (Kaisoon et al., 2012). The leaf of the plant has antifungal and insecticidal effects, containing 26 different bioactive components, of which 86% are reported to be constituted by (Z)-β-ocimene, dihydrotagetone, (Z)-tagetone, limonene, (E)-ocimene, and (Z)-ocimenone (Singh et al., 2003). The plant has been shown to have antioxidant, antimicrobial and antibacterial activities (Kaisoon et al., 2012; Tripathi et al., 2012; Verma and Verma, 2012; Motamedi et al., 2015; Dasgupta et al., 2016), accelerate blood clotting in wound healing (Dasgupta et al., 2016), and exhibit positive effects on vision, moisturizing and protecting skin, and maintaining the normal rhythmic operation of the heart, and for these properties, the plant extract is commercially sold and recommended for drug production due to its phytochemical content (Regaswamy and Koilpillai, 2014). The flowers of the plant are used as a coloring agent in foodstuffs (Barzana et al., 2002), animal feed, and textile products (Jothi, 2008). They have also been proven to contain plenty of lutein, which has increased their value due to their nutritional properties (Hadden et al., 1999). In addition, T. erecta is effectively used in biological control against certain microorganisms that live in plant roots and damage plants, such as nematodes (Natarajan et al., 2006; Tan, 2011) and spiders (Erdoğan, 2017). The current study aimed to investigate the effect of extracts obtained from T. erecta on some microorganisms that cause vaginal discharge outside the menstrual cycle, to determine the effect of these extracts on some microorganisms that cause urinary tract infections, to investigate gels and pads produce with these extracts in order to achieve their practical and widespread use, and to raise awareness of women about vaginal discharge.

MATERIALS AND METHODS

Study material

T. erecta L. was used as material in the study. The fresh leaves, stem and flowers of this plant were collected from the campus of Namik Kemal University (Tekirdağ, Turkey, 40°59’38.5”N 27°35’08.6”E). The samples taken from yellow- and orange-flowering plants were packed separately. With assistance from the botanic department of the university, the collected samples were confirmed to be
**T. erecta L.**

**Drying of plant samples**

The collected samples were washed under tap water, and then sterilized with a 5% alcohol (isopropyl alcohol) solution. The sterilized leaves, flowers and stems were dried at room temperature for 10-15 days (Verma and Verma, 2012). The dried samples were powdered and stored in airtight containers.

**Preparation of T. erecta L. extracts**

The magnetic stirring extraction method was used in the preparation of the extracts. Three groups were formed from the prepared plant samples. The first group contained 5 g yellow flowers + 5 g orange flowers, the second group consisted of a leaf and stem mixture of 5 g each, and the third group comprised 2.5 g of material from each sample. Samples of 10 g were placed in a glass beaker, to which 100 ml of 80% ethyl alcohol (8: ethyl alcohol, 2: distilled water) was added, and the beaker was placed in a magnetic stirrer and mixed at room temperature for 24 h (Hingmire Yashashri, 2017). After the extract was filtered, it was dried by evaporating the solvent in the desiccator.

**Preparation of media**

The media to be used in determining the effects of the extracts on microorganisms were prepared as follows:

- **Nutrient Agar (NA):** After dissolving 20 g powdered medium with distilled water, it was completed to 1,000 ml volume and sterilized in the autoclave at 121°C for 15 min at 1 atmospheric (ATM) pressure.

- **MRS Broth:** After dissolving 52.2 g powdered medium in 1,000 ml of distilled water, it was distributed in cotton tubes and sterilized in the autoclave at 121°C for 15 min at 1 ATM. For the preparation of MRS agar, MRS broth was weighed for 1,000 ml, and then 15 g of agar was added and sterilized in the autoclave.

- **Mueller Hinton Broth (MHB):** 21 g of powdered medium was completed to 1,000 ml with distilled water, distributed into cotton tubes, and sterilized in the autoclave at 121°C for 15 min at 1 ATM.

- **Mueller Hinton Agar (MHA):** 34 g of powdered medium was completed to 1,000 ml with distilled water and sterilized in the autoclave at 121°C for 15 min at 1 ATM.

- **Sabouraud Dextrose Broth (SDB):** 30 g of powdered medium was completed to 1,000 ml with distilled water, distributed into cotton tubes, and sterilized in the autoclave at 121°C for 15 min at 1 ATM.

- **Sabouraud Dextrose Agar (SDA):** After 65 g of medium was dissolved with distilled water, it was completed to 1,000 ml and sterilized in the autoclave at 121°C for 15 min at 1 ATM. After the agar medium was sterilized with the autoclave, it was cooled to 45-50°C and poured into petri dishes.

**Determination of the antimicrobial activities of the extracts**

The antimicrobial activities of the extracts obtained from the flower, leaf + stem, and flower + leaf + stem of T. erecta were examined with the modified disk diffusion method. For the determination of antimicrobial activities, *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Lactobacillus acidophilus* (ATCC 11975) bacteria and *Candida albicans* (ATCC 90028) yeast strain were used. Bacteria stock cultures were planted in NA (E. faecalis and E. coli) and MRS agar (*L. acidophilus*), and yeast culture in SDA for growth, and they were left to incubate at 37°C for 16-18 h. After incubation, the colonies that grew in the media were sampled and transferred to tubes with MHB, MRS broth and SDB. The turbidity of the suspensions was adjusted according to 0.5 McFarland standards; that is, approximately 108 CFU/ml for the bacteria and 1-5x106 cells for the yeast using a densitometer.

The prepared microorganism suspensions were planted in the medium using a swab. The extracts obtained from the various parts of the plant were sampled at a density of 10 mg/ml using distilled water with the disk diffusion method. Different amounts of the extract samples prepared (25, 50 and 100 µl) were placed in wells opened in solid media. The cultivated plates were left to incubation at 37°C for 16-18 h. Ciprofloxacin (CIP, 5 mcg/disk) was used as a positive control. The study was conducted in triplicate. The diameters of the inhibition zones that formed against the microorganisms were evaluated (Figure 1).

**Gel production with the T. erecta flower extract**

At this stage of the study, a protective gel was produced using the flower extract, which has a higher antimicrobial effect than others. In gel production, in addition to the plant extract, distilled water, propylene glycol (for moisturizing properties), a gelling agent (Carbopol 974P NF), a pH regulator (triethanolamine), and a preservative (Sensiva PA30) were used. First, the gelling agent and distilled water were placed in the same glass container and left for 24 h to rise without mixing. Thereafter, the extract and other substances were added and mixed in the homogenizer at 500 rpm for 10 min until it became homogeneous, and the gel was obtained (Figure 2).
Pad production with the *T. erecta* flower extract

This part of the study was carried out in the research and development department of a commercial company producing pads and diapers. First, experiments were undertaken with acetone, water and ethyl acetate in order to determine which solvent was best to dissolve the extract. The extract was determined to dissolve well in water and ethyl acetate. Then, 1 g of the extract was dissolved in 100 ml of ethyl acetate, and the liquid obtained was applied to...
Figure 2: Protective gel produced using the extract obtained from the T.tages erecta flower.

Table 1: Diameters of inhibition zones (mm) formed by the extracts against the investigated microorganisms.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Plant Part</th>
<th>Name of organism</th>
<th>Zone of inhibition in mm</th>
<th>Inhibition zone with CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>25 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>1</td>
<td>Flower</td>
<td>E. coli</td>
<td>11.3±0.57</td>
<td>16.6±1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. fecalis</td>
<td>15.3±0.57</td>
<td>18.0±1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. asidophylus</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. albicans</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>2</td>
<td>Leaf + stem</td>
<td>E. coli</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. fecalis</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. asidophylus</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. albicans</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>3</td>
<td>Flower + leaf + stem</td>
<td>E. coli</td>
<td>22.3±1.52</td>
<td>26.0±0.0</td>
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<tr>
<td></td>
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<td>E. fecalis</td>
<td>20.6±0.57</td>
<td>22.0±0.0</td>
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<tr>
<td></td>
<td></td>
<td>L. asidophylus</td>
<td>15.3±1.15</td>
<td>16.3±0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. albicans</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard error mean (n = 3).

the pads with syringe and spraying methods.

Investigation of women’s knowledge of vaginal discharge

Before and during the study, short interviews were undertaken with married and single women of different ages from different educational backgrounds living in different areas. In these interviews, it was discussed whether they had any knowledge of vaginal discharge, whether they visited a doctor when they encountered such problems, how they found solutions, and whether they were aware of the problems this discharge could cause if left treated.

Statistical analysis

Statistical analyses were performed using Jump statistical package program. All the results were expressed as mean ± standard error mean.

RESULTS AND DISCUSSION

The antimicrobial activities of the three different amounts of three different extracts obtained from T. erecta were investigated in E. faecalis, E. coli and L. acidophilus bacteria and C. albicans yeast strain. As a result of the inhibition zone measurements carried out using the disk diffusion method, it was observed that the flower + leaf + stem extract had an antibacterial effect on E. faecalis, E. coli and L. acidophilus but the leaf + stem extract did not have an antibacterial effect on these bacteria. It was determined that the flower extract showed antibacterial activity especially against E. faecalis and E. coli, and it was effective in L. acidophilus at a concentration of 100 µl (Table 1). This result is very important for our study because L. acidophilus plays an important role in the balance of the vaginal flora. An excessive decrease in the number of L. acidophilus would lead to changes in pH, multiplication of other bacteria, and development of vaginal discharge (Kaygusuz et al, 2014). Considering that the extracts that do not affect this microorganism but have high antibacterial activities on the remaining bacteria would be more suitable for our
purpose, we decided to use the flower extract in gel and pad production.

Previous studies have shown that *T. erecta* is effective in *E. coli* (Chakraborty, 2009; Das and Mishra, 2011; Verma and Verma, 2012; Ramha and Madhavan, 2011; Behidj-Benyounes et al., 2014; Motamedi et al., 2015; Padalia et al., 2015; Salehi et al., 2018), which is also supported by our findings. Although *E. coli* is found in normal body flora, when it increases in number, it causes urinary tract infections, inflammation of the intestines, and wound formation where it accumulates. Therefore, the extract we obtained can also be used in the treatment of urinary tract infections and tissue damage that may occur as a result of itching due to vaginal discharge.

The extract obtained from the leaves of the plant was tested on *E. faecalis* only in one previous study, and it was found to be slightly effective (Dasgupta et al., 2012). This bacterium that causes infection is found in adult reproductive cavities, vagina, gallbladder, digestive system, and urinary tract. They are directly responsible for intraabdominal infections and pelvic and urinary tract infections. In addition, since they live in the reproductive organs, they cause the deterioration of the vaginal flora and formation of discharge and urinary tract infections. Problems are encountered in their treatment because they are resistant to many antibiotics and can quickly develop resistance against new antibiotics. Therefore, as in our study, alternative methods for conditions caused by *E. faecalis* should be researched and developed. On the other hand, our review of the literature showed that no study has investigated the antimicrobial effect of *T. erecta* on *L. acidophilus*. Therefore, this study may be the first to investigate the effect of *T. erecta* extracts on this bacterium, which is important for the vaginal flora and causes problems at too high or low quantities.

In this study, we did not determine an inhibitory effect of the extracts obtained from *T. erecta* on *C. albicans*. In contrast, many studies have reported that the *T. erecta* extract has an antifungal effect on this yeast (Chakraborty, 2009; Behidj-Benyounes et al., 2014; Padalia et al., 2015; Salehi et al., 2018). This difference can be attributed to the changes in the active ingredients of the plant depending on the harvest season, development stage, geographic area, and climate, natural or cultural form of the plant, and methods and doses used (Burts, 2004). Previous studies were generally carried out in countries where this plant grows naturally. However, in our study, we used the cultured form grown in Turkey for ornamental purposes.

Interviews conducted with women during the study process showed that most experienced vaginitis (for different reasons), and when this problem was encountered, they did not generally consult a specialist or used any medication. The majority of women were not aware of the importance of this problem for health and that it could lead to uterus, ovarian and other cancers if frequently recurring and left untreated (Breshears et al., 2015). Vaginitis is usually attempted to be treated with vaginal tablets, creams, antibiotics, and antiparasitics, but some of these drugs are not reported to be effective (Balci and Capar, 2005; Kaygusuz et al., 2014). Another greatest problem encountered today is the resistance of microorganisms to antibiotics. At the same time, most drugs used are synthetic and affect many organs such as the stomach, kidney, and liver. Therefore, while seeking alternative solutions for the treatment of vaginal problems, care should be taken to ensure that such treatment only affects the infected area and it is natural. The literature contains many studies on *T. erecta*, but to the best of our knowledge, there is no research exploring solutions to problems such as vaginal discharge and urinary tract inflammation. As a result of our study unique nature, we proposed two different methods for the treatment of vaginitis. The first was a gel produced using the flower extract from *T. erecta*, which can be easily applied to the desired area and only affects that area. There is no other study that produced a gel for this purpose using the extract of this plant. Our second contribution was the production of a pad using the flower extract. The research and development department of a pad manufacturing company performed trials to absorb the extract into pads using spraying and syringe methods, and obtained successful results. In addition, it was determined that this extract could be used in the production of toilet paper, daily pads, and other pads with the spraying method, and it would be even more practical if utilized in the production of Spunbond non-woven interlining, which is the material used to cover the top surface of the pads. Currently, when manufacturing these surface materials, various antibacterial and fragrance chemicals are used.

*T. erecta*, which was proposed as an alternative natural solution against vaginitis in the current study, was previously shown to be used in many areas, including wound healing, accelerating blood clotting, and skin protection due to its antioxidant, antibacterial and antifungal properties (Kaisoon et al., 2012; Tripathi et al., 2012; Verma and Verma, 2012; Motamedi et al., 2015; Dasgupta et al., 2016). The extract obtained from this plant is also commercially available and recommended for pharmaceutical production due to its phytochemical content (Regaswamy and Kolipillai, 2014). Therefore, the commercial importance of *T. erecta*, which is mostly grown as an ornamental plant, is gradually increasing. This situation can also be turned into an advantage for farmers that can grow this plant in suitable areas as a source of additional income.

Vaginitis, which was explained in detail in this study, constitutes one of the most common problems among women, although it is not often discussed. However, it is known that it has an increased incidence in recent years with diseases transmitted by sexual intercourse. The reasons for this increase may differ, but the most important factors are the socio-economic status of societies,
increasing migration from villages to cities and other countries, changing living conditions, easier transportation means increasing and domestic and foreign tourism, starting sexual activities at an early age, ensuring freedom of sexual intercourse due to family planning, and poor hygienic conditions of public areas (toilets) (Karaman et al., 2006). It is not possible to implement solutions to prevent most of these factors in a short time. However, the present study showed the importance of raising women's awareness about this condition as one of the priorities.

CONCLUSION

When vaginal discharge is encountered, natural alternatives should be prioritized over synthetic drugs that damage some organs in the body. Natural, practical and inexpensive treatment methods or measures can be applied with the gels and pads proposed in the present study. These gels and pads can be effective against not only vaginitis but also other conditions such as urinary tract infections caused by the investigated microorganisms.

REFERENCES


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