Antimicrobial activity of Asteraceae species against bacterial pathogens isolated from postmenopausal women

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Abstract

Purpose

Investigation of the antibacterial action of aqueous extracts of *Bidens sulphurea*, *Bidens pilosa*, and *Tanacetum vulgare*, species of Asteraceae family that are popularly used for the treatment of genito-urinary infection.

Methods

The minimum inhibitory concentration (MIC) and minimal bacterial concentration (MBC) of the extracts against standard strains of *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC25922), and *Pseudomonas aeruginosa* (ATCC27853) and against bacteria that were isolated from cultures of vaginal secretions and urine from menopausal women with a diagnosis of recurrent urinary tract infections (rUTI) were determined by broth microdilution.

Results

The MIC values of the three extracts against Gram-positive and Gram-negative standard bacterial strains ranged from 7.81 to 125.00 mg ml⁻¹, and the MBC values ranged from 7.81 to 500.00 mg ml⁻¹. However, *B. sulphurea* was more efficient. In the urine samples, the three extracts inhibited the growth of coagulase-negative *Staphylococcus* spp., and the *B. pilosa* was the most active extract against *E. coli* compared with the other ones. For the
vaginal secretion samples, no significant differences in the inhibition of coagulase-positive *Staphylococcus* spp. and *P. mirabilis* were found among the extracts. *T. vulgare* and *B. sulphurea* were more effective in inhibiting coagulase-negative *Staphylococcus* spp. compared with *B. pilosa*. *E. coli* was more susceptible to the *B. sulphurea* extract compared with the *B. pilosa* and *T. vulgare* extracts.

**Conclusion**

The present results suggested the potential medicinal use of Asteraceae species, especially *B. sulphurea*, as therapeutic agents against rUTI-related bacteria.

**1. Introduction**

Urinary tract infections (UTIs) are the most common bacterial infections in women, and their incidence rises in the postmenopausal period mainly because of lower estrogen production [1]. Among the types of UTIs, recurrent urinary tract infections (rUTIs) are one of the most common problems in urology. Recent studies indicated that rUTIs should be considered as different from primary UTIs [2].

Among the main causative microorganisms of rUTIs are aerobic Gram-negative bacteria that are present in the intestinal microbiota, including members of the Enterobacteriaceae family, such as the genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, and *Shigella* [3]. In community-acquired UTIs, *Escherichia coli* accounts for approximately 85% of cases. In chronic infections and hospital- or structure-related anomalies of the urinary tract, there is a more equitable distribution of different enterobacteria, with a higher prevalence of UTIs that are caused by *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., and Gram-positive *Staphylococcus saprophyticus* and *Enterococcus* spp. [4].

The bacterial resistance of microorganisms that are isolated from human urinary infections is well recognized, resulting in a reduction of therapeutic efficacy, making such treatments ineffective and expensive, prolonging the course of the disease, increasing the incidence of complications, and increasing the mortality rate [5]. Thus, the lack of new therapeutic agents to replace those that have become ineffective has necessitated the search to discover new alternatives to treat UTIs more effectively.

Medicinal plant-based antimicrobials for the treatment of UTIs are a vast source of potential medications, such as the Asteraceae family, which comprises nearly 1,600 genera and 23,000 species [6]. Among the main species of this family that are popularly used for the treatment of genito-urinary tract and bacterial infections are *Bidens sulphurea* (Cav.) Sch. Bip., *Bidens pilosa* L., and *Tanacetum vulgare* L. [7–9].

*B. sulphurea*, popularly known as yellow cosmos or “cosmo-amarelo,” “picão-grande,” and “aster does México,” is an annual herbaceous species from Mexico, but it is considered invasive and intensely disseminated and naturalized in Brazilian territories. It is traditionally used in Brazil to treat bacterial infections and kidney and bladder inflammation [10–12]. *B. pilosa*, popularly known as “picão-preto,” “pica-pica,” and “amor-de-mulher” [7], is a small, annual, erect plant that is native to South Africa and widely distributed throughout the world [7,13]. This species is traditionally used in Brazil for the treatment of bacterial infections, inflammation, and genito-urinary infections [7–9]. *T. vulgare*, popularly known as “catinga-de-mulata,” is a perennial native plant that is widespread in Europe and western Asia [14]. It is widely used...
in Brazilian folk medicine for the treatment of bacterial infections, cystitis, and renal infections [8,14].

Despite the popular use of these species for the treatment of bacterial infections and genitourinary tract infections, the therapeutic activity of these species has not yet been investigated. The present study evaluated the potential antimicrobial activity of aqueous extracts that were obtained by infusions of these species, as recommended by popular use, against bacteria that were collected from urine samples and vaginal secretions from postmenopausal women with a diagnosis of rUTI.

2. Materials and methods

2.1. Patient recruitment

Prior to the collection of clinical samples, the study received approval from the Ethics Committee on Research Involving Human Beings of UNIPAR (CAEE no. 90949218.2.0000.0109). The participants provided both verbal and written consent for urine and vaginal secretion collection for research purposes. Women in the postmenopausal period (45–70 years old) with a diagnosis of rUTI (three episodes of UTI in the previous 12 months or two episodes in the last 6 months) were included in the study [15]. Women with structural genetic abnormalities in the genital or urinary systems, genital dystopias greater than Pelvic Organ Prolapse Quantification stage 2 [16], genetic or drug-induced immune deficiency, neurological deficiency that affected urinary tract function, or malignant pelvic disease or women who had already undergone pelvic radiotherapy and used antibiotics in the last 4 weeks at the time of sample collection were excluded from the study [17].

2.2. Collection of clinical samples

A total of 15 urine samples and 15 samples of vaginal secretions were collected using sterile containers and swabs that contained Aimes medium with activated charcoal (Transystem™, Copan Italia, Brescia, Italy), respectively. The samples were obtained from 15 patients who attended a private clinical routine from May to July 2018. Each participant was given a printed sheet outlining the details of the method to be used for urine collection. It was instructed to wash their hands and clean the genital area with soap and water, discard the first jet and collect the mid-stream urine specimens in a sterile container. Immediately after collection, the samples were sent to the Laboratory of Preventive Veterinary Medicine and Public Health of UNIPAR, Brazil. The samples were transported under ice-cold conditions.

2.3. Identification of clinical samples

Urine samples were seeded on plates that contained Mannitol Salt Agar and MacConkey Agar and incubated at 37°C for 24 h to isolate Gram-positive and Gram-negative aerobic bacteria. The swabs were first placed in tubes that contained 3 ml of Brain Heart Infusion (BHI) medium and incubated in an oven at 37°C for 24 h. Afterward, they were seeded on plates according to the procedure for urine samples. Subsequently, macroscopic and microscopic analyses and biochemical tests were performed [18]. Gram-positive, catalase-positive cocci underwent a coagulase assay to classify coagulase-positive and coagulase-negative Staphylococcus. The Enterobacteriaceae family was biochemically identified using a set of biochemical tests in the Enterobacteria Kit (NewProv, Paraná, Brazil) according to the manufacturer’s instructions.
2.4. Determination of susceptibility to antimicrobials

Antibiotic resistance and susceptibility of the identified organisms were determined using the disc diffusion method [19] with commercially available discs of metronidazole (50 μg/disc), amoxicillin (10 μg/disc), norfloxacin (10 μg/disc), and oxacillin (10 μg/disc). The samples were thawed and added to the culture medium to grow each isolate and incubated. After growth, the bacterial inoculum was padronized on the McFarland 0.5 scale and seeded on Mueller Hinton agar using a sterile swab. After 15 min, the antimicrobial-impregnated disks were incubated at 37˚C for 18–24 h. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones (in millimeters; including the 6.5 mm disc diameter) for each of the microorganisms. The inhibition zones were measured in triplicate.

2.5. Multiple antibiotic resistance index

The multiple antibiotic resistance (MAR) index of each strain was calculated according to the formula of Krumperman (1983) [20]: 

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\frac{a}{b}
\]

where \( a \) is the number of antibiotics to which a particular isolate was resistant, and \( b \) is the total number of antibiotics tested.

2.6. Plant material and extract preparation

Botanical material (\( B. \) sulphurea and \( B. \) pilosa) was obtained from the Medicinal Garden of Paranaense University (UNIPAR; S23˚47’55”, W53˚18’48”), Umuarama, PR, Brazil. One specimen of each species was registered in the Medicinal Garden of Campus 2 of UNIPAR (no. 131 and 40, respectively). Aerial parts of \( T. \) vulgare were collected in the Municipal Nursery of Saudade do Iguaçu, PR, Brazil (S25˚41’30.069”, W52˚37’06.207”), and an exsiccate was deposited in the Herbarium of the Federal Technological University of Paraná of Dois Vizinhos, PR, Brazil (no. DVPR006294). All of the species were collected in May 2018. The aqueous extracts of the plants were obtained by infusion as recommended by popular use [12] with minor modifications. The dried and ground vegetable material (100 g, the material was pulverized in a knife mill until granulometry of 850 μm) was subjected to an extraction process by infusing with 1 L of boiling water. Extraction was performed until the extraction medium reached room temperature (24 hours). The residue was separated by filtration, and the supernatant was resuspended in ethanol (1:3 extract/ethanol) for the precipitation of proteins and polysaccharides, obtaining a precipitate and an ethanolic supernatant from the infusion (48 hours of precipitation). After complete removal of the organic solvent by rotavaporation (3 hours/400 mL; 45˚C), the extract was subjected to lyophilization (72 hours; -42˚C). The final yields of the extracts of \( B. \) sulphurea, \( B. \) pilosa, and \( T. \) vulgare were 21.43%, 17.45%, and 23.13%, respectively. The extracts were stored in a freezer until use.

2.7. Gas chromatography/mass spectrometry

The chemical constituents of the extract samples were identified using a gas phase chromatograph (Agilent 7890 B) coupled to a mass spectrometer (Agilent 5977 A) equipped with an Agilent HP-5MS UI capillary column (30 m × 0.250 mm × 0.25 μm). For the analysis, an injection volume of 1.0 μl of a solution that was prepared by the dissolution of 20 mg of the \( B. \) sulphurea, \( B. \) pilosa, and \( T. \) vulgare extracts in 1.0 ml of methanol was used. The analytical conditions were the following: 280˚C injector temperature operating in split mode (1:2), 280˚C transfer line, and 1 ml min⁻¹ carrier gas (helium) flow. The initial column temperature was 80˚C (1 min) with a ramp of 2˚C min⁻¹ until reaching 185˚C. The temperature remained at 185˚C for 1 min, followed by heating at 9˚C min⁻¹ until reaching 275˚C. The temperature remained at 275˚C for 2 min, followed by heating at 25˚C min⁻¹ to 300˚C. The temperature
was then held at 300˚C for 1 min. The extracts of *B. sulphurea*, *B. pilosa*, and *T. vulgare* underwent electron impact ionization scanning at 70 eV with a 40–600 mass/charge ratio (m/z). The ionization source temperature and quadrupole temperature were 230˚C and 150˚C, respectively. The compounds were identified by comparing their mass spectra with the NIST 11.0 library and by comparing the retention indices (IRs) that were obtained by the homologous series of n-alkane standards (C7-C28; [21]).

2.8. Minimum inhibitory concentration and minimum bactericidal concentration of extracts

The minimum inhibitory concentration (MIC) and minimal bacterial concentration (MBC) of the extracts against standard strains and bacteria that were isolated from cultures of vaginal secretions and urine were determined by the broth microdilution method using Mueller Hinton Broth according to the CLSI [19] with modifications. The vegetal extract was dissolved in Tween 80 (2%) and diluted in culture medium to an initial concentration of 500 mg mL\(^{-1}\). Then, serial decimal dilutions (1:2) were prepared by adding culture medium to achieve concentrations ranging to 0.97 mg mL\(^{-1}\). Thus, a final volume of 100 μL (culture medium plus extract) was distributed in 96-well plates, as well as controls of culture medium and culture medium with extract and Tween. Bacteria were standardized on the McFarland 0.5 scale and the inoculum adjusted to ~ 10\(^5\) CFU/mL. The tests were performed in triplicate and the plates incubated at 37˚C for 24 hours. Readings were performed after the addition of 10 μl of 10% diluted 2,3,5-triphenyltetrazolium chloride, followed by incubation at 37˚C for 30 min. Bacterial growth was considered when the wells presented any pink tone after incubation [22]. The MIC was the lowest concentration of the extract that inhibited bacterial growth. The MBC was determinate by subculturing 10 μL from the culture of each negative well on Mueller Hinton Agar plates as described above [23].

2.9 Statistical analysis

Differences between groups were assessed using analysis of variance (ANOVA), followed by Tukey’s post hoc test. Values of \(p < 0.05\) were considered statistically significant. The results are expressed as mean ± standard error of the mean (SEM). The statistical analyses were performed using Statistica 13.3 software.

3. Results

3.1. Effect of antibiotics on bacterial pathogens and their MAR index

Thirty-two bacterial samples were isolated from 15 postmenopausal women who were diagnosed with rUTI, predominantly from urine (10; 31.25%) and vaginal secretions (22; 68.75%). Gram-positive (*Staphylococcus* spp.) and Gram-negative (*Escherichia coli* and *Proteus mirabilis*) bacteria were identified in urine and vaginal secretion samples.

The antibiotic resistance of the identified organisms was determined by the disc diffusion method using antibiotics that are routinely used for the treatment of UTIs. Table 1 shows the percentage of urine samples and vaginal secretions that were antibiotic-resistant. Overall, we observed multidrug-resistant isolates, with a majority from vaginal secretions (48; 57.83%) and urine (18; 51.42%).

The MAR index results indicated that all of the tested genitourinary tract isolates of *Staphylococcus* spp. (19 isolates), *E. coli* (11 isolates), and *P. mirabilis* (3 isolates) had a very high MAR index (> 0.2; Table 2), indicating that the samples were classified as high risk.
3.2. Chemical composition of extracts

Table 3 show the gas chromatography profile and probable chemical composition of the B. pilosa, T. vulgare, and B. sulphurea extracts, respectively. B. pilosa had β-sitosterol (22.33%, C_{29}H_{50}O, Mw = 414.39) as the most abundant compound, ethyl iso-allocholate (17.52%, C_{26}H_{44}O_5, Mw = 436.31), artepillin (12.84%, C_{20}H_{20}O_8, Mw = 388.00), β-carotene (12.51%, C_{40}H_{56}, Mw = 536.00), followed by betulin (11.18%, C_{30}H_{50}O_2, Mw = 442.38), decanoic acid 1,1α,1β,4,4α,5,7α,7b,8,9-decahydro-4α,7b-dihydroxy-3-[hydroxymethyl]-1,1,6,8-tetramethyl-5-oxo-9α-H-cyclopropa[3,4]benz[1,2-e]azulene-9α-diylester, [1αR

Table 1. Antibiotic resistance (R) percentage of Gram-positive bacteria (Staphylococcus spp) and Gram-negative bacteria (Escherichia coli, Proteus mirabilis) isolated from urine samples and vaginal secretion of menopausal patients with a diagnosis of genitourinary tract infection.

| Antibiotics      | Urine samples | Vaginal secretion | Urine samples + Vaginal secretion |
|------------------|---------------|-------------------|----------------------------------|
|                  | R | % | R | % | Total (R) | % |
| Gram-positive bacteria |
| Amoxicillin      | 1 | 20 | 4 | 28.57 | 5 | 27.77 |
| Metronidazole    | 5 | 100 | 12 | 85.71 | 17 | 94.44 |
| Norfloxacin      | 1 | 20 | 4 | 28.57 | 5 | 27.77 |
| Oxacillin        | 3 | 60 | 9 | 64.28 | 12 | 66.66 |
| Gram-negative bacteria |
| Amoxicillin      | 2 | 40 | 6 | 66.66 | 8 | 57.14 |
| Metronidazole    | 5 | 100 | 9 | 100 | 14 | 100 |
| Norfloxacin      | 1 | 25 | 4 | 44.44 | 5 | 35.71 |
| Total            | 18 | 51.42 | 48 | 57.83 | 66 | 50.38 |

https://doi.org/10.1371/journal.pone.0227023.t001

Table 2. Multiple antibiotic resistance (MAR) index of bacteria isolated from urine samples and vaginal secretion of menopausal patients with a diagnosis of genitourinary tract infection.

| Samples | Gram-positive bacteria | Gram-negative bacteria |
|---------|------------------------|-----------------------|
|         | Urine samples | Vaginal secretion | Urine samples | Vaginal secretion |
| 1       | 0.5          | 0.25           | '0.33        | '0.33          |
| 2       | -            | 1.00           | -            | -              |
| 3       | -            | 0.75           | -            | -              |
| 4       | -            | 0.25           | '1.00        | '1.00          |
| 5       | -            | 0.50           | -            | -              |
| 6       | -            | 0.25           | -            | -              |
| 7       | 1.00         | 0.75           | -            | '1.00          |
| 8       | 0.25         | 0.50           | -            | -              |
| 9       | -            | 1.00           | -            | -              |
| 10      | 0.50         | 0.50           | '0.33        | '0.33          |
| 11      | 0.25         | 0.50           | '0.33        | '0.66          |
| 12      | -            | 0.50           | -            | -              |
| 13      | -            | 0.50           | -            | '0.33          |
| 14      | -            | -              | '0.66        | '0.66          |
| 15      | -            | -              | -            | '1.00          |

Gram-positive: Staphylococcus spp., Gram-negative: *Escherichia coli, *Proteus mirabilis. MAR was calculated according to the method described by Krumpelman [20].

https://doi.org/10.1371/journal.pone.0227023.t002
### Table 3. Chemical composition of *Bidens pilosa*, *Tanacetum vulgare* and *Bidens sulphurea* extract.

| Compounds | R_t (min) | Relative area (%) | m/z | Structural formula | MS |
|-----------|-----------|--------------------|-----|--------------------|----|
| Bidens pilosa | Tanacetum vulgare | Bidens sulphurea |
| 1 | 5-methylheptan-2-amine | 850 | - | - | 19.39 | 129.15 | C_9H_{18}N a, b, c |
| 2 | Costunolide | 1590 | - | - | 6.54 | 232.15 | C_{15}H_{20}O_3 a, b, c |
| 3 | Verrucarol | 1599 | - | 15.61 | - | 266.15 | C_{13}H_{18}O_4 a, b, c |
| 4 | Hexadecanoic acid ethyl ester | 1708 | 2.35 | 3.71 | 3.90 | 270.26 | C_{17}H_{34}O_2 a, b, c |
| 5 | Tridecanoic acid methyl ester | 1781 | - | - | 1.43 | 270.26 | C_{17}H_{34}O_2 a, b, c |
| 6 | Methyl linolenate | 1901 | 1.57 | - | - | 292.24 | C_{19}H_{32}O_2 a, b, c |
| 7 | Octadecanoic acid methyl ester | 1918 | - | - | 1.89 | 294.47 | C_{19}H_{32}O_2 a, b, c |
| 8 | Phytol | 2013 | 1.18 | 14.03 | 5.98 | 296.30 | C_{20}H_{40}O a, b, c |
| 9 | Palustric acid | 2044 | - | - | 0.46 | 302.22 | C_{20}H_{40}O a, b, c |
| 10 | Isohumulone | 2083 | - | - | 8.18 | 362.00 | C_{21}H_{30}O_2 a, b, c |
| 11 | Artemetin | 2090 | 12.84 | 15.74 | 23.28 | 388.00 | C_{20}H_{26}O_8 a, b, c |
| 12 | n.i. | - | - | - | - | - | - |
| 13 | n.i. | - | - | - | - | - | - |
| 14 | Ergosterol | 2810 | t | 6.39 | - | 396.33 | C_{29}H_{44}O a, b, c |
| 15 | Stigmasterol | 2900 | 4.02 | - | - | 412.37 | C_{29}H_{44}O a, b, c |
| 16 | β-sitosterol | 2928 | 22.33 | - | 17.15 | 414.39 | C_{29}H_{44}O a, b, c |
| 17 | Ethyl iso-allocholate | 3018 | 17.52 | 9.31 | 1.86 | 436.31 | C_{28}H_{42}O a, b, c |
| 18 | Betulin | 3074 | 11.18 | 0.58 | - | 442.38 | C_{30}H_{50}O_2 a, b, c |
| 19 | Stigmasteryl acetate | 3115 | - | 7.43 | - | 454.38 | C_{31}H_{52}O_2 a, b, c |
| 20 | n.i. | - | 1.01 | 0.59 | | | |
| 21 | Oleic acid | - | - | 10.19 | 0.82 | 456.36 | C_{28}H_{48}O a, b, c |
| 22 | 9,19-Cyclichloestene-3,7-diol, 4,14-dimethyl-3-acetate | 3220 | - | - | 1.38 | 472.39 | C_{31}H_{44}O a, b, c |
| 23 | 7,8-Epoxylanostan-11-ol, 3-acetoxy- | 3324 | 1.02 | 13.12 | 5.39 | 502.40 | C_{32}H_{50}O_4 a, b, c |
| 24 | β-carotene | 4053 | 12.51 | - | - | 536.00 | C_{40}H_{56} a, b, c |
| 25 | 3,4,3',4'-Tetraydrospiroilxanthin | 4259 | 3.40 | - | - | 600.49 | C_{42}H_{56}O_2 a, b, c |
| 26 | Decanoic acid (1,1a,1b,4a,5,7a,7b,8,9-decyl-4a,7b-dihydroxy-3-[hydroxyethyl]-1,1,6,8-tetramethyl-5-oxo-9a-H-cyclopenta [3,4]benz[1,2-e] azulene-9,9a-diyl ester 1aR-(1aα,1bβ,4aα,7aα,7bα,8α,9αβ,9αα)] | 4263 | 8.53 | - | - | 672.00 | C_{40}H_{48}O a, b, c |
| 27 | n.i. | - | 0.49 | 0.33 | - | - | - |
| Total identified | 98.45 | 97.01 | 97.65 | | | |

*Compounds listed in order of elution in column HP-5MS;*

*R_t = Identification based on retention index using a homologous series of n-alkane C_7–C_{29} on Agilent HP-5MS column.*

*MS = Identification based on comparison of mass spectra using with the NIST 11.0 library.*

Relative area (%): percentage of the area occupied by the compounds in the chromatogram. n.i. = not identified. t = traces. (-) = without compound.

https://doi.org/10.1371/journal.pone.0227023.t003

(1αα,1ββ,4αβ,7αα,7βα,8α,9β,9αα) (8.53%, C_{40}H_{54}O_8, Mw = 672.00), stigmasterol (4.02%, C_{29}H_{48}O, Mw = 412.37), 3,4,3’,4’-Tetraydrospiroilxanthin (3.40%, C_{42}H_{56}O_2, Mw = 600.49), hexadecanoic acid ethyl ester (2.35%, C_{17}H_{34}O_2, Mw = 270.26), methyl linolenate (1.57%, C_{19}H_{32}O_2, Mw = 292.24), phytol (1.18%, C_{20}H_{40}O, Mw = 296.30) and 7,8-Epoxylanostan-11-ol, 3-acetoxy (1.02%, C_{32}H_{50}O_4, Mw = 502.40). *T. vulgare* had arte- metin (15.74%, C_{20}H_{26}O_8, Mw = 388.00), verrucarol (15.61%, C_{15}H_{22}O_4, Mw = 266.15), phytol (14.03%, C_{20}H_{40}O, Mw = 269.30), 7,8-Epoxylanostan-11-ol, 3-acetoxy (13.12%, C_{32}H_{54}O_4, Mw = 502.40), oleanolic acid (10.19%, C_{28}H_{44}O_5, Mw = 456.36), followed by ethyl iso-allocholate (9.31%, C_{28}H_{44}O_5, Mw = 436.31), stigmasterol acetate (7.43%,

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**Note:** The table and its content have been accurately transcribed and formatted for readability. Any further questions or analysis regarding the content can be performed based on this representation.
Table 4 shows the results of the in vitro screening of the antibacterial activity of the aqueous extracts against bacteria that were isolated from urine and vaginal secretion samples from post-menopausal women. In urine samples for coagulase-negative Staphylococcus spp., no significant difference was found between the extracts (F$_{2,12}$ = 1.72, p = 0.21). The one-way ANOVA indicated a significant difference between the extracts against E. coli (F$_{2,9}$ = 4.21, p < 0.05) and P. mirabilis (F$_{2,6}$ = 12.00, p < 0.01). Tukey’s post hoc test showed that the extract of B. pilosa
Table 5. Antibacterial activities of Gram-positive and Gram-negative bacteria isolated from urine samples and vaginal secretion of menopausal patients with a diagnosis of genitourinary tract infection, expressed as minimal inhibitory concentration (MIC, mg mL\(^{-1}\)) and minimal bactericidal concentration (MBC, mg mL\(^{-1}\)).

| Microorganisms | Bidens pilosa | Tanacetum vulgare | Bidens sulphurea |
|----------------|--------------|-------------------|-----------------|
|                | MIC          | MBC              | MIC            | MBC          | MIC          | MBC          |
| **Urine Sample** |              |                   |                |              |              |              |
| Staphylococcus spp. Coagulase - | 37.88±22.26a | >31.25            | 18.74±5.29a     | >62.50       | 3.17±1.29a   | >15.62       |
| E. coli         | 58.59±24.18a | >250.00           | 125.00±0b       | >125.00      | 78.12±15.62a,b | >62.50       |
| P. mirabilis    | 166.66±41.66a| >250.00           | 125.00±0a       | >125.00      | 5.85±1.95b   | 7.81         |
| **Vaginal secretion** |          |                   |                |              |              |
| Staphylococcus spp. Coagulase + | 78.12±46.87a | >31.25           | 8.78±6.83a     | >62.50       | 2.43±1.46a   | >15.62       |
| Staphylococcus spp. Coagulase - | 37.63±14.30a | >31.25           | 8.78±2.75b     | >62.50       | 3.14±0.66b   | >15.62       |
| E. coli         | 102.67±14.80a| >250.00           | 116.07±8.92a    | >125.00      | 62.50±0b     | >62.50       |
| P. mirabilis    | 93.75±31.25a | >250.00           | 62.50±0a       | >125.00      | 5.85±1.95a   | >62.50       |

Values are expressed as mean ± SEM. The averages followed by equal letters in the same line for MIC did not differ by the Tukey HSD test (p<0.05).

(58.59 mg ml\(^{-1}\)) was the most active against E. coli (p < 0.05) compared with T. vulgare (125.00 mg ml\(^{-1}\)) and B. sulphurea (78.12 mg ml\(^{-1}\)). B. sulphurea (5.85 mg ml\(^{-1}\)) promoted better inhibition (p < 0.05) of P. mirabilis compared with the extracts of T. vulgare (125.00 mg ml\(^{-1}\)) and B. pilosa (166.66 mg ml\(^{-1}\)).

Coagulase-positive and -negative Staphylococcus spp. was identified in the samples of vaginal secretions (Table 5). No significant difference was found between the extracts in inhibiting coagulase-positive Staphylococcus spp. (F\(_{2,3} = 2.35, p = 0.24\)) and P. mirabilis (F\(_{2,3} = 6.0745, p = 0.08813\)). A significant difference was found between groups in inhibiting coagulase-negative Staphylococcus spp. (F\(_{2,30} = 4.82, p < 0.05\)). Tukey’s post hoc test showed that T. vulgare (8.78 mg ml\(^{-1}\)) and B. sulphurea (3.14 mg ml\(^{-1}\)) were more effective in inhibiting coagulase-negative Staphylococcus spp. compared with B. pilosa (37.63 mg ml\(^{-1}\)).

With regard to inhibiting E. coli, the one-way ANOVA showed a significant difference between the extracts (F\(_{2,18} = 7.80, p < 0.05\). E. coli was more susceptible to the extract of B. sulphurea (62.5 mg ml\(^{-1}\)) compared with the extracts of B. pilosa (102.67 mg ml\(^{-1}\)) and T. vulgare (116.07 mg ml\(^{-1}\)). The MBC values for the clinical samples ranged from 7.81 to >250.00 mg ml\(^{-1}\).

4. Discussion

Infections that affect the genito-urinary tract are caused by Gram-positive and Gram-negative bacteria and are common in both young and old women. Estrogen deficiency plays an important role in the development of bacteriuria [24]. These infections are a serious public health problem because they are recurrent in many patients and can lead to severe sequelae, such as sepsis, pyelonephritis, kidney damage, and premature delivery, and multiresistant strains [2,25]. Urinary tract infections also often result in chronic recurrence, resulting in the frequent use of antibiotics or long-term antimicrobial prophylaxis that exposes patients to the consequences of chronic use of these drugs and long-term changes in normal microbiota of the vagina and gastrointestinal tract [26]. Although rUTIs usually are not life-threatening, the high incidence significantly increases healthcare costs and can negatively impact patients’ quality of life [2].

Based on this alarming growth of uropathogens that are resistant to existing drugs and the side effects of antibiotics, new therapeutic agents that are less expensive and have fewer adverse effects need to be developed [24]. Preventing recurrent genito-urinary tract infections and
improving patients’ quality of life have been the goals of many research groups [2]. Herbal
treatment may be a viable solution for the effective treatment of diseases that are caused by
bacteria [27].
Medicinal plants comprise a large variety of small molecules with antibiotic properties,
especially terpenoids, glycosides, flavonoids, and polyphenols. Most of these small molecules
have poor activity compared with the actions of common antibiotics that are produced by bac-
teria and fungi. However, despite the less potent effects of vegetal derivatives, many plants can
successfully combat infections because of synergistic effects of their different pharmaco-
logically active compounds [28]. In the present study, such synergistic antimicrobial effects of the
crude extracts were observed.
Oral infusion preparations of B. pilosa, B. sulphurea, and T. vulgare are popularly used or
by seat baths [7,12,14]. The present study evaluated the effects of extracts of these plants, pre-
pared by infusion, against bacterial strains that were isolated from urine and vaginal secretion
samples from menopausal women with a diagnosis of UTIs, with the goal of validating their
popular use. Such scientific validation is beneficial for patients because the use of infusion
preparations of these plants in the form of a seat bath to treat UTIs may be associated with
fewer systemic side effects compared with the current antibiotics that are used clinically.
Importantly, medicinal plants are considered low-cost options [29], which would facilitate
patients’ access to such treatment alternatives for UTIs. Such infections are usually recurrent
and present high levels of drug resistance.
In the present study, an elevated MAR index was observed for Staphylococcus spp. (0.25 to
1.00), E. coli (0.33 to 1.00), and P. mirabilis (0.33) that were isolated from urine and vaginal secretion
samples. According to Krumperman [20], a MAR index ≥ 0.2 is observed when
isolates are exposed to high-risk sources of human or animal contamination. Interestingly,
despite the relatively high resistance indices of the studied samples, the extracts effectively
inhibited the growth of coagulase-negative Staphylococcus spp. in urine samples and inhibited
the growth of both coagulase-positive and -negative Staphylococcus spp. in vaginal secretion
samples from menopausal women who were diagnosed with UTIs. This effect was more evi-
dent for the extract of B. sulphurea, with intermediate action of T. vulgare. Coagulase-negative
Staphylococcus is considered a commensal bacteria in humans, and its role as an etiological
agent in various infectious processes has been recognized, especially in urinary infections
[30,31].
In addition to the involvement of Staphylococcus spp. in the development of UTIs, enter-
bacteria are one of the main causes of rUTIs [32]. Bactericidal and bacteriostatic effects of B.
sulphurea species were also observed against bacteria, especially E. coli and P. mirabilis, that
were isolated from urine and vaginal secretion samples from patients with rUTIs. Such an
effect is important because UTIs have a tendency to present recurrence or chronicity, and
more than 85% of these infections are caused by uropathogenic E. coli [33].
In addition to exerting antibacterial activity against Gram-negative (P. aeruginosa and E.
coli) standard strains, a bacteriostatic effect of B. sulphurea was also observed and with lower
intensity in T. vulgare and B. pilosa extracts. Bacteriostatic drugs inhibit the growth of bacteria
in the environment, and actions of the immune system are necessary to eliminate them [34].
In addition to exerting bacteriostatic effects, the previously reported antioxidant effects of
these medicinal plants may also modulate the immune response by increasing interleukin-2,
lymphocytes, and T-cells and decreasing lipid peroxidation and prostaglandin synthesis [35].
Previous phytochemical analysis of B. sulphurea identified phenolic compounds, ferulic
acid, caffeic acid and sesquiterpene lactones [36,37]. In B. pilosa, flavonoids, terpenoids, phen-
ylpropanoids, porphyrins and aliphatic and aromatic compounds are present. [38]. In T. vul-
gare phenolic compounds, terpenoid, caffeoylquinic acid, douglasian, ludovicin and ß-Thujone
can be found [39,40]. The chromatographic analysis of the extracts evaluated in this research indicated that artemetin, a flavonoid with antioxidant effects [41], was a major component of the extracts of B. sulphurea (21%), T. vulgare (11%), and B. pilosa (11%). The antimicrobial activity of artemetin has also been reported in other studies [42,43]. β-sitosterol, a potent antimicrobial phytosterol, was identified in the extracts of B. sulphurea (15%) and B. pilosa (6%) but not in the extract of T. vulgare [44,45]. Additionally, the presence of 5-methylheptan-2-amine, isohumulone, costunolide, tridecanoic acid, and octadecanoic acid methyl ester was only observed in the B. sulphurea extract. The antimicrobial effects of these compounds are well described in the literature [46–49]. The actions of these compounds explain the higher antimicrobial effect of the B. sulphurea extract compared with T. vulgare and B. pilosa.

5. Conclusion

The ethnomedicinal form of Bidens pilosa, B. sulphurea and Tanacetum vulgare preparation presented antibacterial activity against standard strains and bacteria that were isolated from cultures of vaginal secretions and urine from menopausal women with a diagnosis of recurrent urinary tract infections. However, B. sulphurea was more efficient. Thus, these results suggest the potential medicinal use of an Asteraceae species, B. sulphurea, as a therapeutic agent against bacteria that cause rUTIs.

Acknowledgments

The authors thank Ana Karina Vargas Soares, Ailton da Cruz Melo, and Ricardo Ferreira da Silva for their assistance with the experiments and UNIPAR and Fundação Araucaria for providing the research grant and fellowships that enabled this study.

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