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Antimicrobial Original Research Paper

Effects of a new combination of plant extracts plus D-mannose for the management of uncomplicated recurrent urinary tract infections

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Urinary tract infections (UTIs) are an economic burden for public health. The increasing prevalence of resistant bacteria which cause UTIs may be related to the inappropriate prescription of antibiotics. The aim of this preliminary study was to evaluate whether three different combinations of plant extracts plus D-mannose are effective in preventing the recurrence of UTIs. Three groups of patients received three combinations of plant extracts in conjunction with D-mannose. These were: berberine, arbutin and birch (group A); berberine, arbutin, birch and forskolin (group B); and proanthocyanidins (group C). The clinical recurrence of cystitis at the end of treatment and during follow-up was determined by comparison with baseline measurements using the microbiological assessment of urine samples, vaginal swabs and vaginal smear slides. Patients in groups A and B had a lower incidence of episodes of recurrent cystitis during treatment and follow-up, samples with a significantly lower median bacterial load and a reduction of the grade of lactobacillary flora compared to patients in group C.

Keywords: Plant extracts, Recurrent urinary tract infections, Cystitis, Prevention, D-mannose

Abbreviations: UTIs, urinary tract infections, cAMP, cyclic adenosine monophosphate, UPEC, uropathogenic *Escherichia coli*, GFR, glomerular filtration rate, CFU, colony-forming units, CLSI, Clinical and Laboratory Standards Institute, LBG, lactobacillus grade

1. Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases and almost exclusively caused by bacteria.1 According to the latest data, UTIs affect nearly half of all women, and it is estimated that around 11% of women aged over 18 have an UTI each year.[2–4] It is noteworthy that UTIs are common in ambulatory patients and are second only to lower respiratory tract infections in being responsible for hospitalization.[5,6] UTIs are generally classified according to clinical symptoms, laboratory parameters and microbiological data. In addition, they are usually divided into two groups: complicated and uncomplicated infections.[7] This distinction is useful from a clinical management standpoint and it has been used to guide the choice and duration of antimicrobial treatment. In particular, uncomplicated UTIs occur in patients with a structurally and functionally normal urinary tract. In contrast, complicated UTIs are characterized by functional or anatomical abnormalities within the urinary tract.[8] Recurrent UTIs occur more commonly in healthy women who have anatomically and functionally normal urinary tracts.[9] In uncomplicated cases, recurrent UTIs are treatable with a short-course of antibiotics, but the inappropriate use of these antimicrobial agents has been accompanied by the rapid spread of resistant bacterial strains.[10] The prevalent pathogenic bacteria that cause UTIs are Gram-negative pathogens and the most frequently isolated bacterial strain is *Escherichia coli*, representing >80% of infections. Other pathogens commonly associated with uncomplicated UTIs include *Staphylococcus saprophyticus*, *Klebsiella species*, *Proteus mirabilis* and *Enterococcus faecalis*.[11,12] Although the use of antibiotics is the prescription of choice for treatment or prophylaxis of UTIs, there is substantial evidence that plant-based therapies and/or herbal combinations have the potential to improve several complications associated with multiple chronic conditions, including recurrent UTIs.[13–15] These effects could be also explicated by the inhibition of biofilm formation.[16]

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Over the last decade, cranberry has been used to prevent UTIs, primarily among generally healthy women prone to recurrent UTIs. The major anti-adhesive constituents of cranberry have been identified as proanthocyanidins. Nevertheless, numerous in vitro studies have revealed that that cranberry proanthocyanidins inhibit adherence of UPEC P-type to uroepithelial cells[17,18] and recent literature supports the view that cranberry is ineffective for the prevention or treatment of urinary infections. Indeed, a 2012 review of 24 studies with a total of 4473 participants concluded that cranberry juice cannot be recommended for the prevention of UTIs.[19,20] In addition, in 2016, a randomized clinical trial among older women residing in nursing homes demonstrated that the administration of cranberry capsules vs. placebo resulted in no significant difference in the presence of bacteriuria plus pyuria during the course of 1 year.[21] Furthermore, a scientific study by the European Food Safety Authority (EFSA),[22] which addressed the scientific relationship between the proanthocyanidins (PAC) from the cranberry fruit (Vaccinium macrocarpon Aiton) and its ability to defend against bacterial pathogens in the lower urinary tract, concluded that the evidence provided was insufficient to establish a cause and effect relationship. This opinion confirmed that provided by the EFSA in 2009, when new human data didn't provide any additional scientific information which could be used to substantiate the use of cranberry for the treatment or prevention of UTIs.

Recently, alternative prophylactic methods to reduce the rate of recurrent UTIs have also included treatments with D-mannose. This sugar has a number of important roles in human metabolism, particularly in the glycosylation of specific proteins. In vitro studies have shown that D-mannose binds to type-1 pili of enteric bacteria, blocking their adhesion to uroepithelial cells. D-mannose also reduces bacteriuria levels in in vivo animal models and lowers the risk of UTIs in women with a history of recurrent cystitis.[23–26] As long as thirty years ago, herbs containing the alkaloid berberine were shown to be important as microbial anti-adhesive agents.[27,28] However, to date, no clinical trials have examined whether the administration of berberine-containing plants can prevent or alter the course of cystitis. Nevertheless, it has been shown that berberine decreases the expression of fimbriae by *E. coli*, hence preventing their adhesion to the bladder epithelium. Berberine also blocks the adhesion to the urothelium of *Streptococcus pyogenes*, and interferes with the lipoteichoic acid complexes that allow the adhesion of streptococcus to fibronectin.[27,28] In addition, *Arctostaphylos uva ursi* (also known as bearberry) extracts are traditionally used in North America as urinary antimicrobial botanicals. The leaves of this small shrub contain high levels of the phenolic glycoside arbutoside, also known as arbutin. The prophylactic effect of an uva-ursi extract standardized to arbutin was evaluated in women with recurrent cystitis. This double-blind, prospective, randomized trial reported that uva-ursi exerts a prophylactic effect on recurrent cystitis at the end of a one-year follow-up period.[29] Birch (*Betula* sp.) is also known for its antimicrobial activity and its extract has been commonly used in the treatment of UTIs to prevent vaginal colonization caused by uropathogens.[30,31] Considering the key role of cyclic adenosine monophosphate (cAMP) in the inhibition of uropathogenic *E. coli* (UPEC) invasion, several studies have shown that forskolin, a diterpene isolated from the Asiatic herb *Coleus forskohlii*, increases intracellular cAMP and expels UPEC from the intracellular reservoir, increasing the susceptibility of bacteria to immune responses.[32,33]

The aim of this study was to test whether three different orally administered preparations containing combinations of the above-mentioned extracts (berberine, uva-ursi, birch and forskolin) in conjunction with D-mannose, were synergistically effective in preventing the recurrence of UTIs. A commercially available preparation of proanthocyanidins (PAC) plus D-mannose was considered as a comparison group.

2. Material and methods

2.1. Study design

A randomized three-arm parallel group intervention trial was designed to evaluate the prophylactic effects of three plant-based oral formulations combined with D-mannose on female subjects with a history of recurrent UTIs presenting with uncomplicated cystitis. Of 74 eligible women, two were excluded because they did not meet the inclusion criteria (Fig. 1). Thus, 72 women were randomly allocated to three groups as follows: group A ($n = 24$) treated with berberine, arbutin, birch and D-mannose; group B ($n = 24$) treated with berberine, arbutin, birch, forskolin and D-mannose; and group C ($n = 24$) treated with proanthocyanidins and D-mannose. The duration of the intervention was 12 weeks and the end of follow-up was 24 weeks after the start of treatment. All participants attended regular clinical appointments at the start of treatment and 4, 8 and 12 weeks thereafter in order to monitor general health and collect biological samples. Follow-up visits were scheduled at 20 and 24 weeks after the start of treatment. The acute events of cystitis have been treated with antibiotics as per investigator's choice on the basis of his/her clinical judgement but also considering the eventually isolated bacterial species.

All the study procedures were carried out in accordance with the Declaration of Helsinki. The study protocol and the informed consent form were approved by the Independent Ethical Committee for Non-Pharmacological Clinical Trials on 1 February 2014 and registered in the ISRCTN registry (ISRCTN13017713). All subjects provided written informed consent prior to the initiation of any study-related procedures. After giving informed consent, subjects were randomly divided into three groups. We randomly assigned eligible patients to treatment groups after stratification according to centre, in a three-group

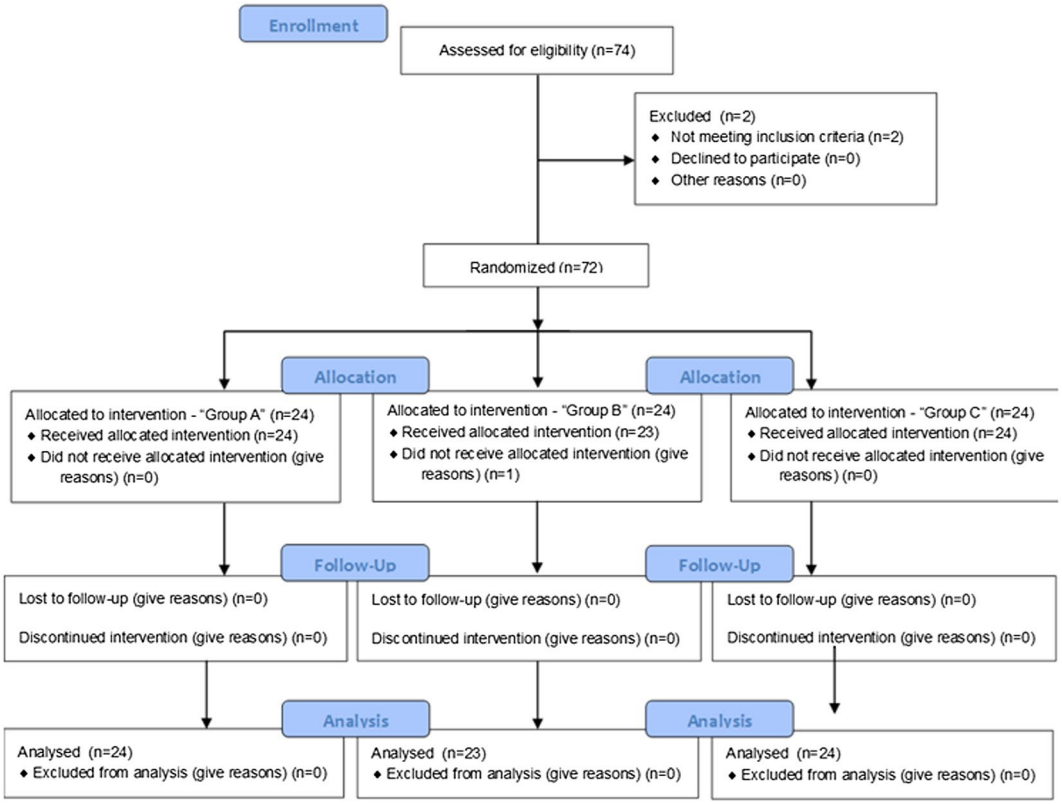

Figure 1 Flow diagram of enrolment, intervention allocation and data analysis.

parallel design. Of every six randomly selected patients, two were allocated to group A (berberine, arbutin, birch and D-mannose), two were allocated to group B (berberine, arbutin, birch, forskolin and D-mannose) and two were allocated to group C (proanthocyanidins and D-mannose).

2.2. Subjects

Eligible subjects were all adult Caucasian females affected by acute uncomplicated cystitis and all had a history of recurrent UTIs. The participants were enrolled (recruitment and treatment) between March 2014 and December 2014 and randomly assigned to the three different groups.

The inclusion criteria were as follows: (1) two or more episodes of UTI in the last 12 months documented by urine culture; and (2) at least one urinary symptom and/or positive urinary nitrate test or leukocyturia. Women were ineligible if any of the following criteria were present: (1) pregnancy or lactation; (2) abnormalities of the upper urinary tract, including the presence of urinary stones; (3) a permanent urinary catheter; (4) complete urinary incontinence; (6) stage 5 chronic kidney disease (glomerular filtration rate (GFR) < 15 ml/min). The study took place at Castiglione Prestianni Hospital area in Bronte (CT), Italy.

2.3. Compounds administration

All test compounds were commercially available dietary supplements marketed with several brands such as: DUTY, CISTIBELL, FEMINAX, FEMISTINA, GENTELLE. For this study DUTY and DUTY^{S}, (Elytra Pharma s.r.l., Milan, Italy) contain the following ingredients, respectively.

Arctostaphylos Uva-Ursi Dry Extract (385 mg), Betula pendula Dry Extract tit. 2.5% in Flavonoids (55 mg), Berberis Aristata Dry Extract (45 mg) and D-mannose (420 mg) – (group A); Arctostaphylos Uva-Ursi Dry Extract (385 mg), Betula pendula Dry Extract tit. 2.5% in Flavonoids (55 mg), Berberis Aristata Dry Extract (30 mg), Coleus Forskohlii Dry Extract tit. 10% (30 mg) and D-mannose (420 mg) – (group B); Cistiflux plus containing type-A proanthocyanidins (36 mg) and D-mannose (500 mg) (group C). This last group was included in order to provide a comparison with a widely used supplement. Subjects belonging to groups A and B received two tablets daily, consumed in the morning (D-mannose) and in the evening (extracts). Subjects allocated to group C received one tablet daily consumed in the evening, which contained proanthocyanidins and D-mannose. All patients were contacted by telephone and asked to attend a check up after the start of prophylaxis. Compliance with treatment was assessed by means of product accountability in the following way: at each appointment, the expected amount of consumed capsules was compared with the amount dispensed minus the amount of the product the subject returned. The primary outcome was to evaluate the clinical recurrence of cystitis at the end of treatment and at follow-up compared to that at the start of treatment. Additional outcome measures included the microbiological assessment of urine samples, vaginal swabs and vaginal smear slides to determine the reduction of uropathogens.

2.4. Laboratory assessments

Urinalyses and cultures were performed using standard methods, as previously described.[34] Urinalyses were performed either by microscopy or by dipstick, and urine culture by susceptibility testing. The presence of leukocytes, erythrocytes, epithelial cells and casts was detected by urine microscopy. Diagnosis of UTIs was based on 10^5 colony-forming units (CFU) in 1 ml of urine sample. Urinary specimens were streaked for isolation on MacConkey agar and blood agar plates for bacterial separation. Positive urine culture results, Gram staining and morphology of pre-dominant colonies were defined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Vaginal swabs were collected and transported using the Amies medium to the Laboratory of the Department of Biomedical and Biotechnological Sciences, University of Catania. Bacteria were identified using colony morphology and Gram stain and biochemical tests were performed using standard microbiological procedures.[35] Vaginal smears were prepared by rolling vaginal swabs across a microscope slide. The status of vaginal lactobacillary flora was assessed using the *Lactobacillus* grade (LBG), a standardized scoring method. The flora were scored as normal (LBG I), intermediate (LBG IIa and LBG IIb) and abnormal (LBG III).[36] The smears were also Gram stained and the following lactobacillus morphotypes were detected microscopically: large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*Gardnerella vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* species morphotypes), curved Gram-variable rods (*Mobiluncus* species morphotypes) and Gram-positive cocci.

2.5. Statistical analysis

Clinical data (bacterial count) were analysed by the non-parametric Kruskal–Wallis – One-Way Analysis of Variance on Ranks. Linear regression analysis of the individual counts was carried out using the mean CFU as the dependent variable and time (d) as the independent variable. p value < 0.05 was considered statistically significant.

3. Results

The study included a total of 72 women with acute cystitis and a history of recurrent cystitis episodes. Seventy-one participants completed the 12-week intervention period; 24 in group A, 23 in group B and 24 in group C. Only one of the participants in group B dropped out of the study before the intervention period ended. The treatment was well tolerated and no adverse side effects events were observed.

The Table 1 shows the number of symptomatic episodes recorded during the observation period starting from week 4 and, since we ascertained that the number of recurrences does not correlate to the urine count at the enrolment, we avoided to insert the raw count results at enrolment in the table to not affect the synoptically view. In detail, during the study period, four (16.66%) women in group A had episodes of UTI. Of these, two of them had two episodes each, while another two women experienced a single recurrence during the study period. In group B, there were no episodes of recurrence of UTIs during the intervention period and only two subjects (8.69%) recorded a single episode of cystitis during the follow-up. The number of patients who had a recurrence in group C was 7 (29.16%) and the episodes of UTI occurred during and after treatment (Table 1).

At enrolment, 35% of the collected urine samples had a pathological bacteriuria ($>10^5$ CFU/ml), 40% of women had asymptomatic bacteriuria with a microbial count ranging from 10^2 to 10^5 and the remaining 25% had negative or $< 10^2$ CFU/ml. The bacterial count was evaluated in all treatment groups and also in individuals with a lower cut-off level of $\geq 10^5$ CFU/ml.. The urine collected by the patients at the various control visit were checked in the appearance and, the individual strains from urine culture were isolated and characterized, and the susceptibility test was performed. But, since it is a preliminary study with a short observation period and a low number of recurrence occasions, in order to not jeopardize the purpose of this work, we have chosen to not show the counts of individual isolated strains with relative susceptibility test, but rather the total count. A dedicated study will be planned to deepen the microbiological aspect of the study. Fig. 2 shows the trend of the bacterial counts of urine cultures and the corresponding regression line. A stable trend was observed in group A ($R^2 = 0.0265$) and a reduction was seen in group B ($R^2 = 0.1787$). The rate of bacteriuria in patients belonging to group C was unaltered during the 12 weeks of treatment but an increase in the average values during follow-up ($R^2 = 0.5528$) was observed. Moreover, at the end of the study, the percentile distribution of the

Table 1 Number of patients with recurrent episodes of cystitis during the study.

Group	Patient N°	4 weeks	8 weeks	12 weeks	20 weeks	24 weeks
A	2	–	–	–	1	1
	1	–	–	–	–	1
	1	–	–	–	1	–
B	1	–	–	–	1	–
	1	–	–	–	–	1
C	1	1	1	–	1	1
	1	–	1	–	1	–
	1	–	–	–	1	1
	2	–	–	–	–	1
	2	–	–	–	1	–

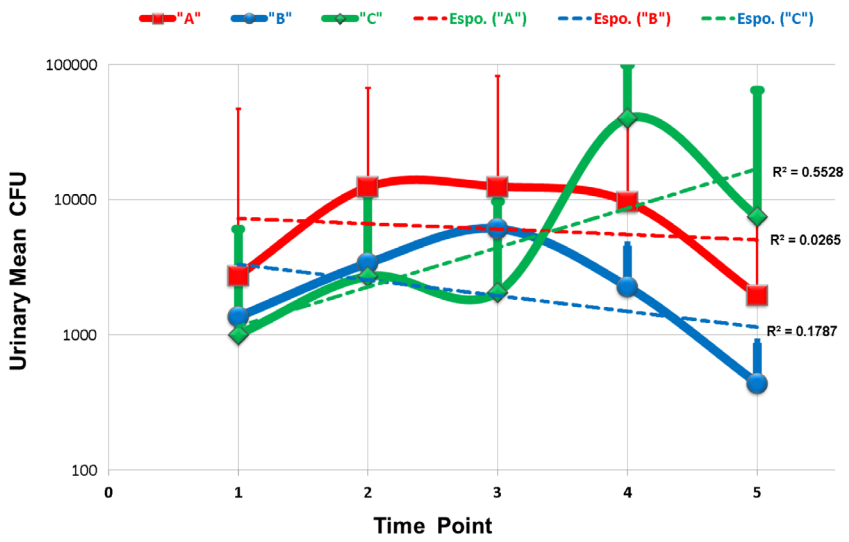

Figure 2 Trend showing the bacterial count during the study period. The comparison between positive and negative urine samples shows that, after 24 weeks, the median bacterial load of urine cultures in groups A and B was stable or lower compared to the beginning of treatment. An opposite trend was observed in patients belonging to group C. Statistical analysis: Kruskal–Wallis – One-Way Analysis of Variance on Ranks; $p = 0.046$ A vs. C.

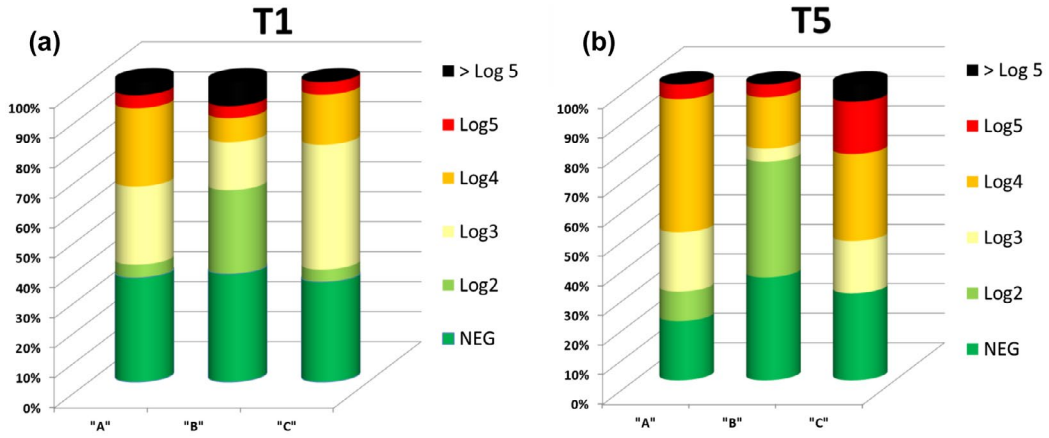

Figure 3 Graphical representation of the percentile distribution of microbial count in urine cultures at enrolment visit (T1) (A) and at the end of follow-up (T5) (B). At the enrolment time, most of the collected urine had a pathological bacteriuria (microbial count ≥ 10^5 CFU); after 24 weeks the groups A and B showed a major number of negative urine cultures vs. C.

microbial count in urine cultures appeared different compared with the beginning of the study period (Fig. 3(A) and (B)). Groups A and B showed a greater number of negative urine cultures compared to group C. Indeed, a higher microbial count (10^5 and/or $>10^5$ CFU/ml) was more frequently observed in the urine cultures of group C compared to groups A or B. The influence of the treatment on the presence of lactobacilli in the vagina was also assessed by analysing vaginal swabs. During the study period, all groups showed an increase in negative vaginal swabs and this result was more evident in group B compared to groups A or C (Fig. 4). In addition, the presence of Lactobacillus morphotypes in the vaginal flora was evaluated by LBG. Fig. 5 shows a marked reduction in all groups. In particular, patients belonging to group B exhibited a more pronounced decrease compared to groups A and C. Of note, group C showed small differences in the LBG score at the beginning and at the end of the study.

4. Discussion

Over the last 10 years, combined administration of botanicals or individual botanical treatments have been associated with health improvement and disease prevention.[37–41] In this context, phytomedicine research has become a key area for the prevention of recurrent UTIs.[20,42] UTIs remain a huge burden for our healthcare systems and the increasing prevalence of antibiotic resistance among uropathogens presents a major challenge to the clinical management of UTIs.[43–45] Therefore, new treatment options and the development of new antimicrobial approaches to prevent recurrent UTIs, particularly those attributable to the establishment of recalcitrant intracellular bacterial reservoirs, are needed. In this study, we evaluated the synergistic efficacy of an innovative composition containing antimicrobial herbs in the management of uncomplicated recurrent UTIs. There have been no clinical trials evaluating combinations of the compounds

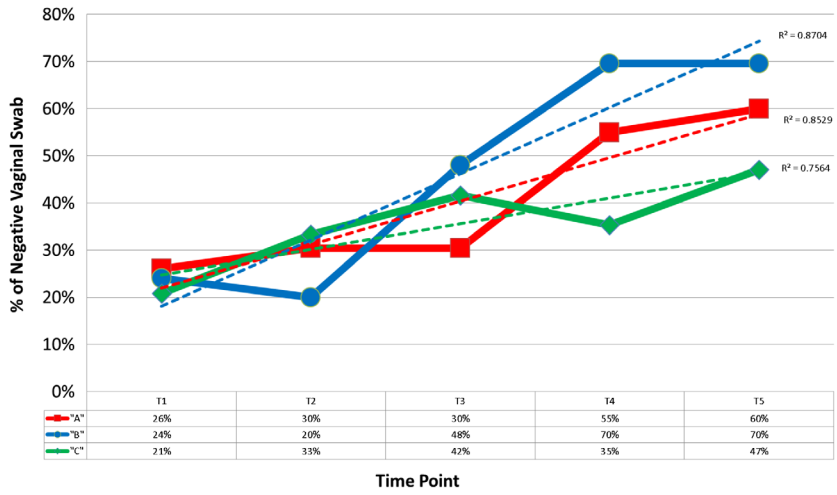

Figure 4 Average of negative vaginal swabs collected from patients monitored during the 24 weeks of the study. T1: visit before treatment; T2: 4 weeks; T3: 8 weeks; T4: 12 weeks; T5: follow-up. All treatments showed a gradual increase of negative vaginal swabs.

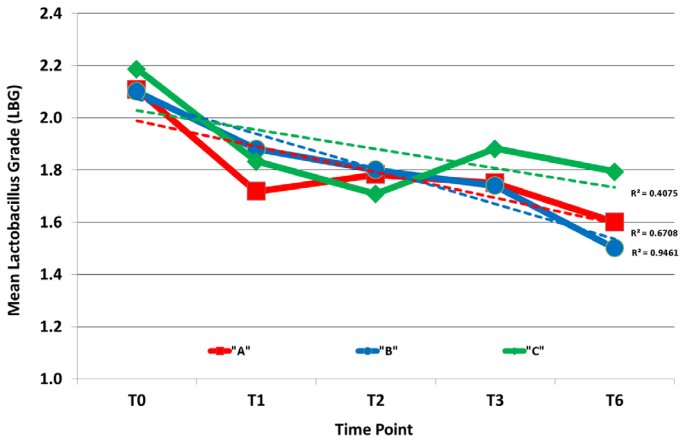

Figure 5 The presence of *Lactobacillus* morphotypes was evaluated according to *Lactobacillus* grade. The average score shows a more pronounced decrease in the group B compared to group A and C.

used in this study, but the potential preventive role of the single extracts used in these formulations have been previously evaluated.[46] To our knowledge, this is the first human study specifically conducted to investigate the effectiveness of berberine, arbutin, birch and forskolin combined with D-mannose in female adults affected by uncomplicated cystitis and a positive history of recurrent cystitis. Our study shows that oral administration of these compounds reduces the incidence of recurrent episodes of cystitis during treatment and follow-up, compared to patients in group C treated with proanthocyanidins and D-mannose (Table 1). This result highlights the possibility that the effectiveness of this treatment may be due to the specific characteristics of each component, but their combination may amplify their effects. It is also important to draw attention to the fact that several studies suggest D-mannose is an effective prophylactic agent and much evidence has shown its ability to inhibit bacterial adherence to uroepithelial cells.[47–49] We also found that berberine, arbutin, birch and forskolin in conjunction with D-mannose reduced the prevalence of positive urine cultures during the study period. In contrast, we observed an increase in the rate of bacteriuria in patients treated with proanthocyanidins and D-mannose during follow-up (Fig. 2). This difference is probably due to the fact that the extracts used to treat subjects belonging to group B promote long-term anti-adherence activity against various uropathogenic strains. In addition, as the vagina is a key anatomical site in the pathogenesis of urinary tract infection (UTI),[50–56] changes in the vaginal microbiota may result in the loss of normally protective *Lactobacillus* spp. increasing the risk of UTI Furthermore, restoring the altered vaginal microbiota equilibrium, i.e. following protracted antibiotic administration, could reduce the risks of UTI recurrences.

Thus, according to these observations, we determined whether the use of these new oral preparations exert some measurable effect on the vaginal microflora.

We observed an increase in the number of negative vaginal swabs recorded, which was particularly evident in group B during the study period (Fig. 4). We also found that the treatments improved the vaginal microflora and reduced the LBG score in all the intervention groups. However, group B showed a more pronounced decrease compared to groups A and C (Fig. 5). This was an unexpected finding given that oral supplementation with herbal compounds and D-mannose cannot be expected to change and modulate vaginal microflora dominated by lactobacilli. The strength of the present study lies in its high participant retention rate during the intervention and follow-up. This may be due to multiple factors: (1) participants received calls from staff members; (2) the study did not require a significant amount of training or time investment; (3) the delivery form (oral, one or two tablet/day) allowed high compliance and consequent high retention rate. However, although highly promising, results of this study have several limitations. Firstly, one of the major limitations was the limited number of enrolled patients. Secondly, the study was not blinded. Moreover, considering the paucity of clinical studies, no information concerning the efficacy of the herbal components in decreasing UTIs was extrapolated. Despite this, there is a growing need to develop and test novel alternative strategies to reduce antibiotic consumption and the incidence of recurrent UTIs. Although 12 weeks of treatment were enough to enable a reduction of recurrent cystitis episodes, another potential limitation of our study was the relatively short-term treatment and follow-up. These data must be considered as preliminary and will require future investigation in a larger trial. Nevertheless, these findings can be considered consistent with the aims of this study.

5. Conclusions

The present study provides preliminary evidence in support of the hypothesis that a combination of selected botanicals plus D-mannose has the potential for a safe, effective and original approach to the reduction of recurrent UTIs. A replication of the study in larger cohorts, with a deeper investigation of the microbiological aspect including pathogens identification and susceptibility test at each time point, is necessary. Nevertheless, these findings are highly promising for attempts to improve the health of patients with chronic UTIs and to minimize the costs for healthcare structures which result from antibiotic resistance.

Conflict of interest

KM, GT, FV, GS, RDM, are inventors of the granted European patents ep2895180 – 'Composition for the prevention and treatment of acute and recurrent urinary tract infections' without financial or other competing interest.

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Contributors

GC–collected the data. DS analysed the data, wrote the article in part. MK wrote the article in part and revised the article. TC conceived, designed, and revised the article. ND collected the data. CS collected and analysed the clinical data. VF-analysed the data. TE-revised and analysed the clinical data. SG -revised the article. DMR conceived and designed the study, obtained funded.

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