TITLE: Xyloglucan, hibiscus and propolis for the prevention of urinary tract infections. Results of *in vitro* studies

SHORT TITLE: A medical device for the prevention of UTIs

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Summary

Aim: To assess the properties of a medical device containing xyloglucan, propolis and hibiscus to create a bio-protective barrier to avoid the contact of urophatogenic *E. coli* (UPEC) strains on cell walls in models of intestinal (CacoGoblet) and uroepithelial (RWPE-1) cells.

Materials & methods: Two UPEC strains (expressing type 1 fimbriae and P fimbriae) were used to assess by electronic microscopy and ELISA the barrier properties of the medical device. The antimicrobial activity was assessed in broth dilution assays.

Results: The three components (xyloglucan, propolis and hibiscus) did not alter *E. coli* cell integrity in intestinal and uroepithelial cell models and were devoid of antibacterial activity. The three components avoided bacterial contact in both cell monolayers.

Conclusion: The non-pharmacological barrier properties of xyloglucan, propolis and hibiscus confirm the role of the medical device for the management of UTIs.

KEY WORDS: intestinal epithelial cells; urinary tract infection; medical device; xyloglucan; hibiscus, propolis

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent infectious diseases and, in consequence, a widespread health problem, with economical and health care consequences [1-3], mainly affecting women, but also patients with catheters, diabetes, immunodeficiency syndromes, underlying urologic abnormalities, and children [4,5]. The primary causative agents of UTIs, accounting for greater than 80% of these infections, are strains of uropathogenic *Escherichia coli* (UPEC) [6], the majority of which ascend from the intestine through the urethra and the bladder and, sometimes, to the kidneys [7].

After colonization, the next step in the pathogenesis of a UTI is the adhesion of uropathogens to the epithelial bladder cells. Following adherence, uropathogens are protected from removal by micturition. The adhesion of *E. coli* to the uroepithelial cell receptors of the host is accomplished by hair-like organelles called fimbriae. The most important are type 1 fimbriae and P-fimbriae. Type 1 fimbriae mainly play a role in the pathogenesis of cystitis and P-fimbriae in pyelonephritis [6,7].

Individuals prescribed an antibiotic for a UTI in primary care acquire bacterial resistance to that antibiotic [8]. The WHO global surveillance report highlights an increase in bacterial resistance to fluoroquinolones used to treat UTIs (ineffective in more than 50% of cases in some countries [9].

The alarming increase in antimicrobial resistance is a global threat to future treatment of infections and has stimulated interest in non-antibiotic prophylaxis of recurrent UTIs [1,5,7,10].

In this regard, non-pharmacological oral supplements, including cranberry proanthocyanidins [11-13], probiotics [14] and a medical device containing a mucosal protector -as xyloglucan or reticulated protein-, hibiscus and propolis) [1,10,15], have been evaluated for the prevention of UTIs. Although it is recognized that more research is needed, the use of non-pharmacological products to prevent UTIs should be considered a useful and safe alternative to antibiotics in this era of increasing antibiotic resistance [10,14].

The medical device containing xyloglucan, hibiscus and propolis (Novintethical Pharma, SA, Switzerland) is a non-pharmacological oral supplement that was approved recently for the prevention of UTIs. It contains xyloglucan (a natural hemicellulose) as the main ingredient, along with the natural products propolis and *Hibiscus sabdariffa*, known with urinary protective properties and to have a role in preventing UTIs [1,15-17]. Xyloglucan belongs to a new class of products, defined as "mucosal protectors", which form a bio-protective film, restoring the physiological functions of the intestinal walls. Results of recent clinical studies have shown that the administration of xyloglucan is a fast, efficacious and safe option for the treatment of acute diarrhea in adults and children [18,19].

The rationale for the potential preventive action of the medical device in UTIs is based on the protective properties of the ingredients in the intestine to avoid the adhesivity of *E. coli* in the "intestinal reservoir" [10,20], the first step of uropathogenic *E. coli* proliferation which is followed by bacterial migration from the intestinal tract to the perineal region and, therefore, to the urinary tract [10,21-23]. We have also the hypothesis that the components of the medical device are also able to create a barrier that avoids the contact of UPEC on the cells of the urinary tract.

In previous *in vitro* studies, we demonstrated in a model of intestinal mucosa (Caco-2 and CacoGoblet™ cells) that this medical device (1.5 to 10 mg/mL) protected cell tight junctions and protected cells from *E. coli* intracellular invasion, being the first step for the demonstration of the efficacy of this product to prevent UTIs [10].

In a recent randomized, double-blind, placebo-controlled clinical trial in 60 patients with one or more symptoms of UTIs (dysuria, urgency, suprapubic pain and/or urine organoleptic changes), the administration of a similar medical device (containing reticulated protein, hibiscus and propolis) twice daily for 5 days provided greater symptom relief than placebo, with a lower risk ratio of patients needing antibiotic treatment than placebo [1]. In an another double-blind, placebo-controlled clinical trial in adult women with recurrent uncomplicated cystitis, the administration of 1 capsule of the medical device/day during antibiotic treatment and during 2 months post-antibiotic treatment significantly reduced the symptomatic recurrence (by 19.4%) in comparison with placebo ($p = 0.015$), with no recurrence being observed after the first month of follow-up [15].

In this context, the present *in vitro* study has been designed to assess the barrier properties of the ingredients of the medical device (xyloglucan, propolis and hibiscus) against adhesion of UPEC strains in a model of intestinal (CacoGoblet™ cells) and uroepithelial cells (RWPE-1 cells), by means of electronic microscopy and ELISA assays, to demonstrate the mechanism of action of the medical device in preventing UTIs, at both intestine and urinary tract.

2. MATERIALS AND METHODS

2.1. Ingredients of the medical device

We evaluated the effects of the ingredients of the medical device: xyloglucan, extracted from the seeds of the tamarind tree (*Tamarindus indica*) and extracts of *Hibiscus sabdariffa* and propolis. These ingredients were kindly provided by Novintethical Pharma, SA, and diluted in phosphate buffer solution.

We assessed the range of concentrations from 1 to 10 mg/ml for the extracts of *Hibiscus sabdariffa* and propolis, exceeding the maximum possible concentrations of these ingredients in the intestine, faeces or urine that can be achieved with the administration of the product studied. In the case of xyloglucan, given that 200 mg/day can be administered and nothing is absorbed, the concentrations obtained in colon and faeces are from 0.13 to 1 mg/ml. In this case, the range of 1 to 10 mg/ml was also studied.

2.2. Bacterial strains

Two UPEC strains from the Culture Collection of University of Göteborg (CCUG) collection were used: *E. coli* expressing type 1 fimbriae (nº 12 from CCUG) and *E. coli* expressing P fimbriae (nº 41 from CCUG).

Bacteria used in the antibacterial activity assay were: *E. coli* (the two strains from CCUG), *Pseudomonas aeruginosa* (CECT111), *Staphylococcus aureus* (CECT240) and *Enterococcus faecalis* (CECT481).

2.3. Cells and reagents

CacoGoblet™ (Readycell, Spain) cells were used for the intestinal mucosa model. Caco-2 and human goblet mucus secreting cells were seeded at a density of 1.5×10^5

cells/well on 0.4 µM PET transwell inserts (Millipore) in 12-well plates and maintained for 21 days. Cells became confluent at day 6 and reached steady state at day 10. Cellular differentiation was completed at day 21. Microvilli and tight junctions were visible by microscopy during cellular differentiation.

CacoGoblet cells were maintained in DMEM medium with high glucose (Dulbecco's modified Eagle medium, Lonza, Belgium) supplemented with 10% fetal bovine serum (FBS, Lonza, Belgium), 1% Non-Essential Amino Acid (NEAA, Lonza, Belgium), 4 mM glutamine (Lonza, Belgium), 10 mM hepes (Lonza, Belgium) and 1% penicillin-streptomycin (Lonza, Belgium), at 37ºC, 90% humidity and 5% CO_2.

RWPE-1 cells (ATTC® CRL-11609™), derived from normal human prostate epithelium, were used as an uroepithelial model. Cells were seeded at a density of 2.4×10^4 cells/well on 0.4 µM PET transwell inserts (Millipore) in 24-well plates and maintained for 7 days to obtain cell confluence.

RWPE-1 cells were maintained in complete KSFM supplemented with 50 µg/ml BPE, 5 ng/ml EGF and 1% antibiotic/antimycotic mixture.

All assays were performed at neutral pH (7.3).

2.4. Adherence assays

Confluent cell layers in 24-well plates were used. Prior to infection, cells were treated with the three ingredients (extract of *Hibiscus sabdariffa*, extract of propolis or xyloglucan) for 1.5 h at 37ºC, 5% CO_2 and 90% humidity. Bacteria at a final concentration of 10^5/ml were then added and cultures were incubated at 37ºC for 2 h to

allow bacteria to adhere. Non-adherent bacteria were then removed by washing the cells two times with PBS. A MOI (multiplicity of infection) of 100 bacteria per eukaryotic cell was used.

Untreated confluent cell layers with and without bacteria were used as controls. All experiments were performed in triplicates.

2.5. Scanning Electronic Microscopy (SEM)

Samples for SEM had to be dry and conductive. The drying process was carried out preserving the original structure of the sample as far as possible and the sample was coated afterwards with a material that made the sample conductive and allowed it to be observed under the microscope.

After removal of non-adherent bacteria (see above), 2.5% glutaraldehyde in PBS was added and the plate was incubated for 1h in cold. The whole plate was washed twice more with PBS and then put to incubate in PBS in the refrigerator for 0.5 h. The whole plate was washed twice more with PBS and 70% ethanol was added, incubating for 0.5 h at room temperature. Lastly, dehydration was continued to absolute ethanol.

The next day, samples were critical-point dried and then, once mounted on supports for transmission electron microscopy, metallised with gold/palladium. Samples were observed at 10,000 – 20,000 augmentations.

179 **2.6. Bacterial quantification**

180 For each variable, 20 fields were counted, with 10 euckaryotic cells per field (a total of
181 200 cells per variable). Bacterial count was analysed directly by SEM.

182 Quantification of bacteria (*E. coli* expressing type 1 fimbriae and expressing P
183 fimbriae) in the adherence assays with RWPE-1 cells were also performed using
184 Enzyme-Linked ImmunoSorbent Assay (ELISA) using a polyconal IgG antibody
185 against *E. coli* O and K antigens (Anti *Escherichia coli* Antibody, Polyclonal IgG-
186 Biorad 4329-4911, Bio-Rad Laboratories SA, Alcobendas, Spain) diluted 1/200 in PBS.
187 Streptavidin-peroxidase from *Streptomyces avidinni* (Sigma Aldrich Química SA, Tres
188 Cantos, Madrid, Spain) was used as a secondary reagent for the detection of
189 biotinylated antibodies diluted 1/1000 in PBS. Finally, the chromogenic substrate for
190 peroxidise 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma
191 Aldrich Química SA, Tres Cantos, Madrid, Spain) was used and detected at an
192 absorbance of 405 nm.

193

194 Before the immunological reaction, cells were ethanol fixed (70%) and endogenous
195 peroxidases were deactivated with 3% H_2O_2 in 1x PBS for 10 min. Non-specific
196 reactivity was blocked by adding 10% Fetal Bovine Serum(FBS) in PBS.

197

198 **2.7. Antibacterial activity assay**

199 Ingredients were tested for potential antimicrobial activity (bactericidal and
200 bacteriostatic) with the broth dilution assay, using two different culture media, Mueller-
201 Hinton broth and Luria Bertaina (LB). Bacteria tested were *E. coli*, *Pseudomonas*

202 *aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* (4 strains of each), at a
203 final inoculum of 10^5 CFU/ml.

204 Ingredients were dissolved and diluted with culture broth at different concentrations.
205 Hibiscus extract was used at concentrations of 2%, 1% and 0.5%; propolis extrat at
206 0.6%, 0.3% and 0.175% and xyloglucan at 0.5%, 0.25% and 0.125%.

207 Then, each tube was inoculated with a microbial inoculums prepared in the same
208 medium at 10^8 CFU/ml, at a proportion of 1:1000 (inoculum : total volume) to obtain a
209 bacterial concentration of 10^5 CFU/ml. After well-mixing, the inoculated tubes were
210 incubated at 37ºC during 24 hours. Inoculated tubes without the ingredients were used
211 as positive controls and non inoculated tubes containing the ingredients were used as
212 negative controls.

213 Monitoring of growth was performed by measuring absorbance at 600 nm.

214

215

216 **2.8. Statistical analysis**

217 A descriptive analysis of quantitative data was performed (mean and standard
218 deviation were obtained). The Student´s T-test was used to compare results between
219 two conditions. P values lower than 0.05 were considered significant.

220

221 **3. RESULTS**

222 **3.1. Xyloglucan, propolis and hibiscus are not endowed with antibacterial activity**

In the different cell models assessed (CacoGoblet and RWPE-1), we observed that the three components, separately assessed at the maximum concentrations (10 mg/ml for xyloglucan and propolis and 1 mg/ml for hibiscus), did not alter *E. coli* cell integrity, without evidences of bacterial lysis in both bacterial strains assessed (*E. coli* expressing type 1 fimbriae and expressing P fimbriae) (Figures 1A [1-3] and 1B [1-4]). In the case of *E. coli* expressing type P fimbriae incubated treated with hibiscus (1 mg/ml) (figure 1B-3) and propolis (10 mg/ml) (figure 1B-4) it seems that the cellular membranes have some irregularities. We consider that in the first case (figure 1B-3) it is due to the deposition of material and, in the second (figure 1B-4), the left part of the membrane is completely normal and the right must be partially disrupted (by the SEM electrons, it is not well focused). In both cases, morphological characteristics and shape are preserved.

The absence of antimicrobial activity of xyloglucan, propolis and hibiscus was also confirmed in the antibacterial activity tests. At all concentrations assessed for the three components, no effect on the bacterial growth was observed, for all bacteria evaluated (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*). The presence of the three components did not interfer the growth of these bacteria during an incubation period of 24h at 37ºC, with similar absorbances at 600 nm than controls without components.

These results confirm the absence of pharmacological bactericidal or bacteriostatic activity of the three components of the medical device.

3.2. The components of the medical device avoids bacterial adhesion on cell monolayers

By means of observation by SEM, both UPEC strains assessed adhered perfectly to the surface of CacoGoblet and RWPE-1 cells (Figures 2A-F). In RWPE-1 cells we observed adhesion of *E. coli* strains at a higher extent, with the formation of biofilms and adhesion at cytoplasmic level and in on the cell expansions (Figures 3A and B).

With the addition of xyloglucan, at 1 and 10 mg/ml, practically no bacteria were seen adhered to the cell surface (Figures 4A-D). In the case of hibiscus and propolis extracts, we also observed a reduction of adhesion, particularly at the highest concentrations (Figures 5A-D).

In both cell cultures, a statistically significant reduction of the number of cell adhered (for both *E. coli* strains) were observed for the three components in comparison with controls ($p < 0.05$). The reduction was also more important at the highest concentration of the components (Table 1). The highest reduction of adhesion was observed with hibiscus at 10 mg/ml (Table 1).

We also detected an important reduction of bacterial adhesion in RPWE-1 cells assessed by ELISA, particularly at the highest concentration of xyloglucan, hibiscus and propolis (10 mg/ml) (Absorbances for xyloglucan, hibiscus and propolis: 0.3740, 0.2116 and 0.318 vs 0.8796 from the positive control) (Table 2).

DISCUSSION

Nowadays, in the advent of increasing bacterial resistance, prescription of preventive measures to avoid recurrent UTIs, particularly in risk groups, as women or children,

268 represents a priority in the routine clinical practice [10,24,25]. Preventive goals include
269 identification of any correctible anatomical or functional predisposing aberrations,
270 hygiene measures, diet and food supplements as non-antibiotic method of prevention
271 [24,25].

272 In this regard, the medical device from Novintethical Pharma SA, Switzerland, is a
273 non-pharmacological oral supplement recently approved for the prevention of UTIs,
274 containing xyloglucan, propolis and *Hibiscus sabdariffa*, known with urinary protective
275 properties and to have a role in preventing UTIs [1,16,17].

276 In a recent double-blind, placebo-controlled clinical trial in 60 adult patients with
277 symptoms of UTI, the administration of a similar medical device twice daily for 5 days
278 significantly reduced the need of antibiotic treatment in comparison with placebo.
279 Moreover, the administration of the oral supplement was associated with an
280 improvement of all the UTI symptoms/signs, including dysuria, urgency, suprapubic
281 pain and organoleptic changes. These results clearly highlight the role of the medical
282 device as an adjuvant non-pharmacological measure in patients with or at risk of UTIs
283 [1]. Moreover, in women with recurrent cystitis, the administration of the medical
284 device has been shown to significantly reduce the rate of recurrences, a common
285 situation that deserves the use of preventive measures as the administration of non-
286 pharmacological products [15].

287 In this context, the results of the present *in vitro* study have demonstrated the non-
288 pharmacological nature of the three components xyloglucan, propolis and hibiscus,
289 devoid of antibiotic activity (in both microscopic and antibacterial activity assays). We
290 have also demonstrated that the three components are able to create a physical
291 bioprotective film that is able to avoid the contact of UPEC strains in a model of

292 intestinal mucosal cells (CacoGoblet^{TM} cells) and uroepithelial cells (RWPE-1 cells),
293 thus supporting their role in two key steps in the pathogenesis of UTIs: avoiding the
294 persistence of UPEC reservoirs in the lower intestinal tract [10,21-23] and avoiding the
295 urogenital colonization of UPEC, interfering in the process by which uropathogens
296 contact with uroepithelial cell receptors [7].

297

298 It is known that uropathogenic *E. coli*, the primary causative agent of UTIs, can
299 adhere to the uroepithelial cells through adhesive organelles, including type 1, P, and S
300 pili along with Dr adhesins, promoting both bacterial attachment to and invasion of host
301 tissues within the urinary tract. This can provide *E. coli* with a survival advantage,
302 allowing the microbes to better resist detection and clearance by both innate and
303 adaptive immune defence mechanisms [6].

304 Type 1 and P fimbriae are the most important virulence factor associated to
305 adherence to uroephitelial cells, acting synergistically to facilitate bacterial colonization
306 [26]. They bind to mono-mannose and globoseries glycosphingolipids, respectively
307 [27,28], with similar structures arranged in two distinct subassemblies, the tip fibrillum
308 and the pilus rod (more simplified in the case of type 1 fimbriae [29].

309 It has been recently shown that P fimbriae mediate binding between the bacteria and
310 the epithelial cells lining the tubules, while type 1 fimbriae appears to play a role in
311 inter-bacterial binding and biofilm formation in the center parts of the lumen [26].

312 In any case, our results have demonstrated that the presence of components of the
313 medical device is able to interfere in both colonization mechanisms.

314 Therefore, the interference with adhesion observed in our study in the RWPE-1 cell
315 line, a widely used and reproducible model of urinary cells [30-34], indicates the main
316 mechanism by which the medical device is able to avoid UPEC colonization and further
317 invasion, in consonance with the favourable results observed in patients with UTIs with
318 similar products [1,15]. RWPE-1 are non-neoplastic adult human prostatic epithelial
319 cells, which, in contrast with urinary neoplastic cell lines (as bladder, ureter or renal
320 pelvic cells), are being more easily cultured.

321

322 Moreover, the avoidance of adhesion also observed in CacoGoblet™ cells (Co-
323 cultured Caco-2 cells and human goblet mucus secreting cells, a better mimicking of the
324 intestine versus Caco-2 monolayer) [10] also confirm the utility of the medical device to
325 reduce UPEC reservoirs at intestinal level, in consonance with previous recent studies
326 in which we observed that the product created a protective physical barrier on
327 CacoGoblet™ cells, protecting cell tight junctions and protecting intestinal cells from
328 *E. coli* intracellular invasion [10].

329 In the present study we have also observed that xyloglucan is also able to create this
330 protective barrier in a model of urinary cells, and also propolis and hibiscus exerts this
331 effect. Since propolis and hibiscus are systemically absorbed, they can exert these
332 protective effects directly in the urinary tract, thus preventing the attachment of possible
333 uropathogenic bacteria, and, in consequence, the occurrence of UTIs. In the case of
334 xyloglucan, since it is not absorbed, we consider that its protective effects are mainly
335 exerted on the intestinal tract.

336

Therefore, our results support the role of hibiscus and propolis in the prevention of UTIs, in line with the popular knowledge and previous experience of these natural products related to UTIs [16,17].

Propolis is a resinous material collected by bees from exudates and buds of plants, then mixed with wax and bee enzymes. In a double-blind, randomized, cross-over clnical trial in 5 volunteers, the propolis excreted in urine after once daily administration together with cranberry produced a significant bacterial anti-adhesion activity in comparison with placebo in a human T24 epithelial cell-line assay and in the *in vivo Caenorhabditis elegans* model, thus supporting the use of propolis as adjuvant to prevent recurrent UTIs [16].

Moreover, in previous studies, it has been shown that extracts of *Hibiscus sabdariffa* are able to inhibit biofilm production of urinary isolates of *Candida albicans*, thus supporting its anti-adhesive properties at urinary level [17].

In this context, our results confirm the capacity of the component of the medical device to form a protective barrier on the urinary tract. Based on this, further clinical studies could be done to assess the preventive effect of the medical device to prevent UTIs in patients at risk of recurrent UTIs. The use of the medical device to prevent catheter-associated UTIs (CAUTIs) could be also considered, taking in to account the high prevalence of these infections [35] and that, in most cases, CAUTIs are caused by microorganisms from the patient´s own gastrointestinal tract [36].

In conclusion, we have demonstrated the non-pharmacological barrier properties of the components of the medical device on intestinal and uroepithelials cell models, thus confirming the role of this product for the management of UTIs in the routine clinical practice.

DISCLOSURE OF INTEREST: The authors declare no commercial interests which could potentially create a conflict of interest with the contents of this paper.

EXECUTIVE SUMMARY

1. A non-pharmacological oral medical device which was approved recently for the prevention of UTIs, containing xyloglucan (a natural hemicellulose), propolis and hibiscus.
2. Xyloglucan belongs to a new class of products, defined as “mucosal protectors”, which form a bio-protective film, restoring the physiological functions of the intestinal and uroepithelial walls.
3. This *in vitro* study evaluated the effects of the three components avoiding UPEC adherence on intestinal (CacoGoblet) and uroepithelial (RWPE-1) cells.
4. Xyloglucan, propolis and hibiscus did not exhibit antibacterial effects on both cell models and in broth dilution assays, thus confirming the absence of pharmacological effect.
5. Xyloglucan, propolis and hibiscus avoided the adherence of two UPEC strains on intestinal and uroepithelial cells.
6. The observed effects of xyloglucan, propolis and hibiscus support the use of the medical device for the management of UTIs in the routine clinical practice.

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TABLES

Table 1. Adherence quantification by SEM (number of bacteria/cell)

	positive control	xyloglucan*		hibiscus*		propolis*	
		1 mg/ml	10 mg/ml	1 mg/ml	10 mg/ml	1 mg/ml	10 mg/ml
***E. coli* expressing type 1 fimbriae**							
Caco Goblet	42.32 ±7.374	5.22 ±1.940	4.1±1.644	4.6±1.826	3.72±1.591	4.72±1.714	4.0±1.784
RPWE-1	31.44 ±7.085	5.8±1.948	5.04±1.958	4.82± .534	4.08±1.482	4.72±1.471	3.88±1.303
***E. coli* expression P fimbriae**							
Caco Goblet	28.92 ± 7.286	3.2 ± 1.142	2.29±0.804	3.44±1.264	2.48±0.788	3.52±1.232	2.6±0.833
RPWE-1	26.86 ± 6.269	2.94 ±0.998	2.54±0.885	3.16±1.218	2.44±0.951	3.32±1.114	2.52±0.886

*p < 0.05 in comparison with positive control

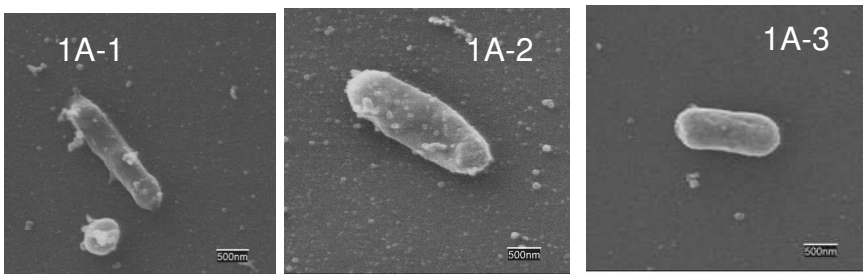
Table 2. Adherence quantification by ELISA (absorbance) (*E. coli* expressing type 1 fimbriae 50% and *E. coli* expressing P fimbriae 50%)

	positive control (without inhibitor)	xyloglucan*		hibiscus*		propolis*		negative control
		1 mg/ml	10 mg/ml	1 mg/ml	10 mg/ml	1 mg/ml	10 mg/ml	
RPWE-1	0.8796 ±0.0075	0.5460 ±0.0295	0.3740 ±0.06	0.3223 ±0.08	0.2116 ±0.05	0.4513 ±0.09	0.318 ±0.099	0.098 ± 0.07

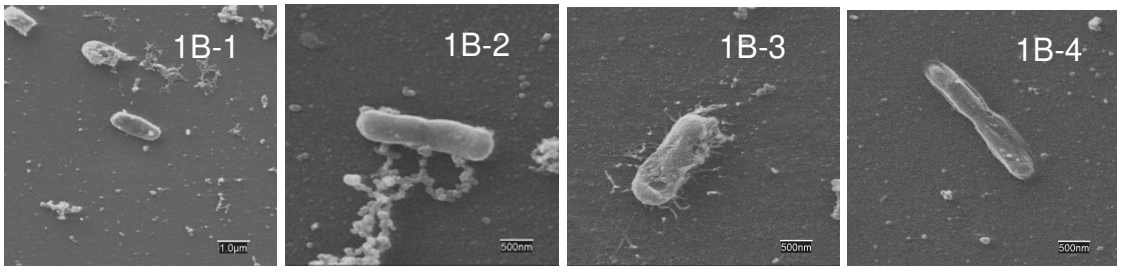
*p < 0.05 in comparison with positive control

FIGURE LEGENDS

Figure 1. Effect of the separated three components (10 mg/ml for xyloglucan and propolis and 1 mg/ml for hibiscus), over *E. coli* cell integrity**:** without evidence of bacterial lysis in both bacterial strains. 1A-1 to 3 and 1B-1 to 4.

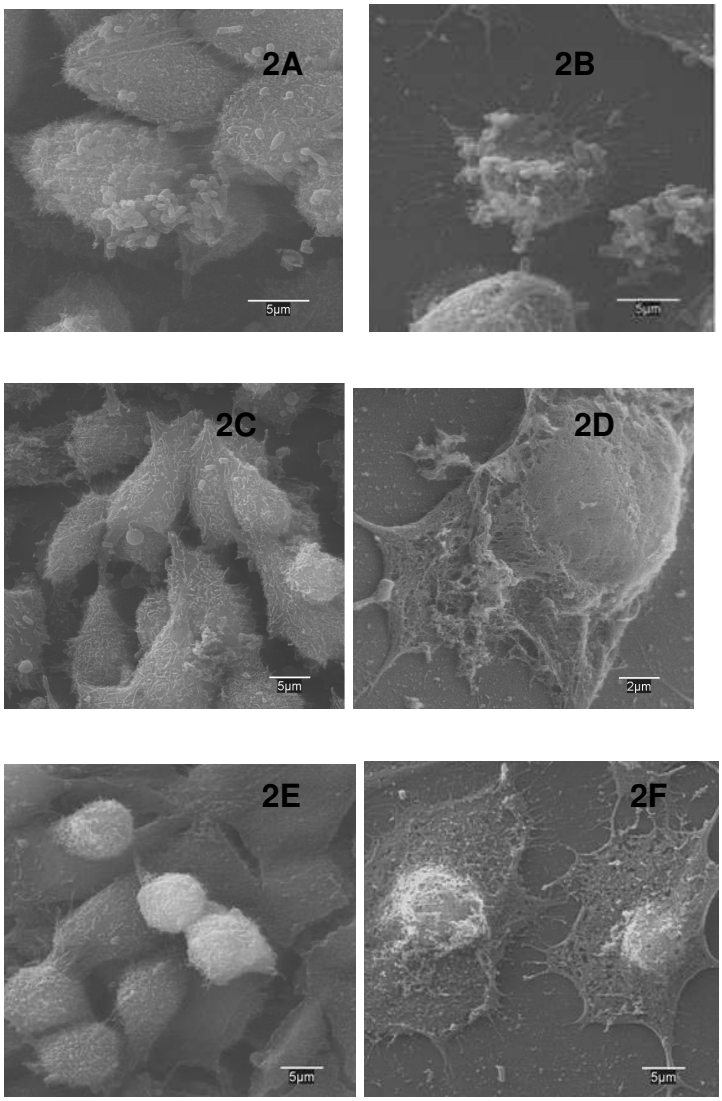


Figures 1A-1 (control *E. coli* expressing type 1 fimbriae); 1A-2 and 1A-3 (Effect of xyloglucan 10 mg/ml and hibiscus 10 mg/ml over *E. coli* expressing type 1 fimbriae.

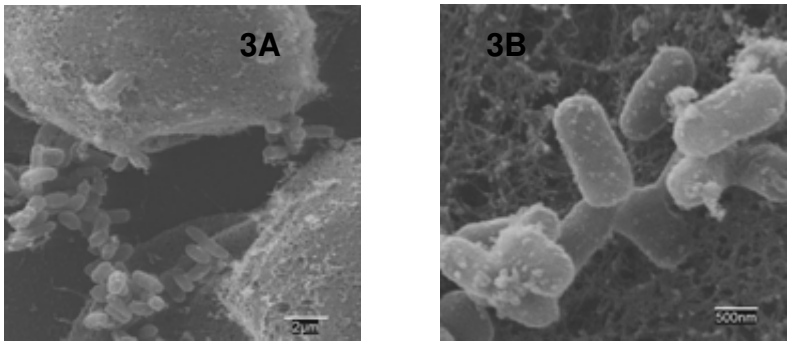


Figures 1B-1 (control *E. coli* expressing type P fimbriae); 1B-2, 1B-3 and 1B-4 (Effect of xyloglucan 1 mg/ml, hibiscus 1 mg/ml and propolis 10 mg/ml over *E. coli* expressing type P fimbriae).

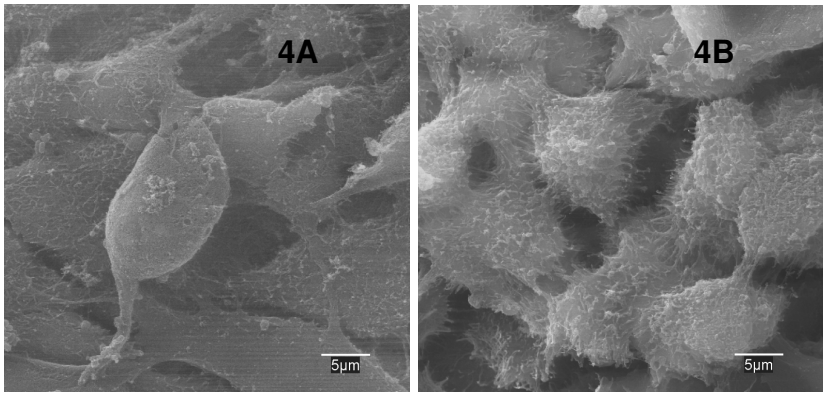
Figures 2. Adhesion of UPEC strains to CacoGoblet and RWPE-1 cells. 2A) Adhesion of *E. coli* expressing type 1 fimbriae to CacoGoblet. 2B) Adhesion of *E. coli* expressing type 1 fimbriae on RWPE-1 cells. 2C) Adhesion of *E. coli* expressing P fimbriae on CacoGoblet. 2D) Adhesion of *E. coli* expressing P fimbriae on RWPE-1 cells. 2E) CacoGoblet cells (without bacterial strains). 2F) RWPE-1 cells (without bacterial strains).

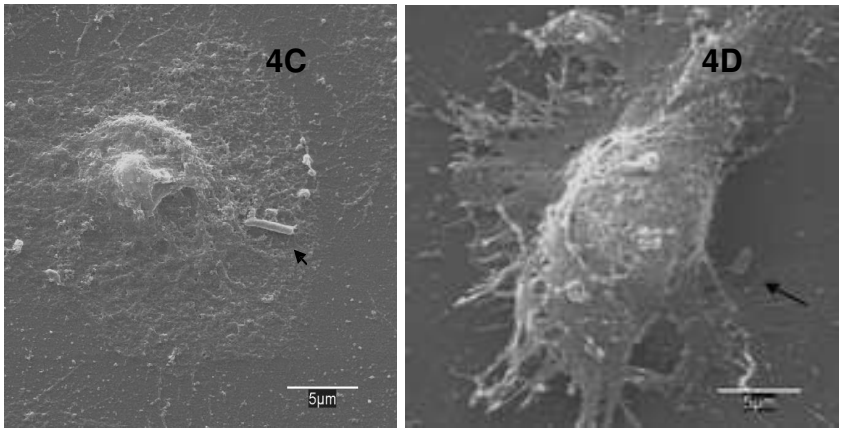


549 **Figure 3.** Adhesion of *E. coli* strains to RWPE-1 cells. A) *E. coli* expressing type 1
550 fimbriae. B) *E. coli* expressing P fimbriae.

551 

552 **Figure 4.** Inhibition effect of xyloglucan (10 mg/ml,) over UPEC adhesion to cell
553 surface. 4A) Adhesion of *E. coli* expressing type 1 fimbriae on CacoGoblet . 4B)
554 Adhesion of *E. coli* expressing P fimbriae on CacoGoblet. 4C) Adhesion of *E. coli*
555 expressing type 1 fimbriae on RWPE-1. 4D) Adhsesion of *E. coli* expressing P fimbriae
556 on RWPE-1.

557 



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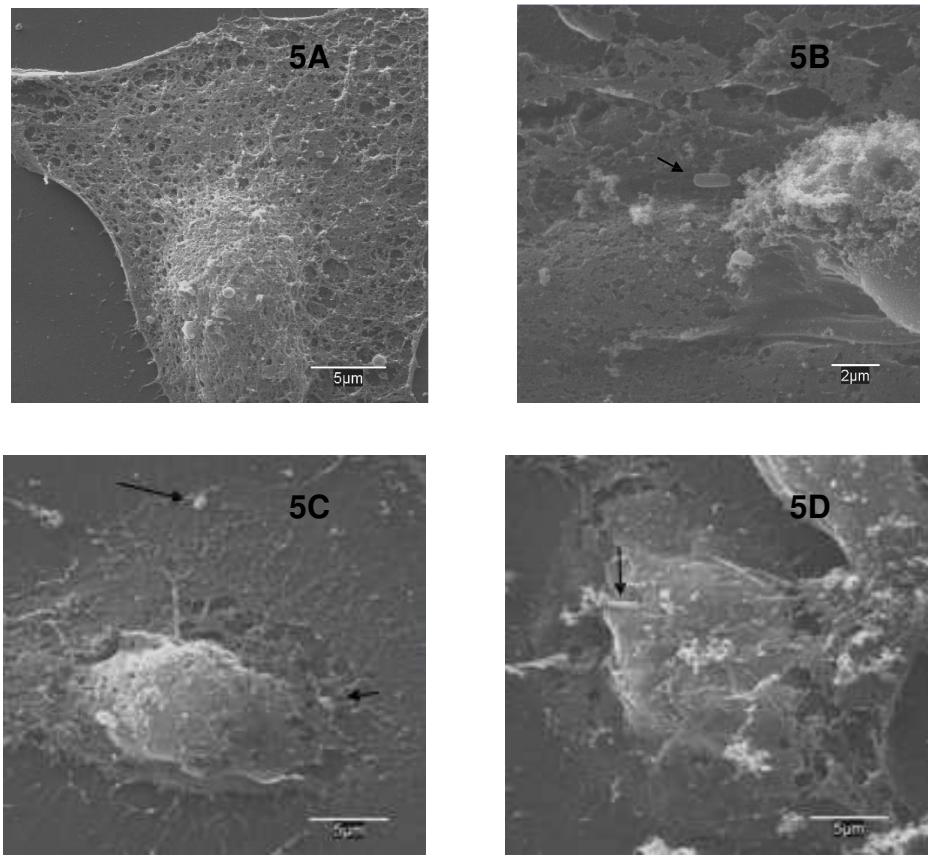
559 **Figure 5.** RWPE-1 Adhesion inhibition. Inhibition produced by propolis (5A) and
560 hibiscus (5B) at 1 mg/ml over *E. coli* expressing type 1 fimbriae; and propolis (5C) and
561 hibiscus (5D) at 10 mg/ml over *E. coli* expressing P fimbriae.



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