APMIS 105: 956–962, 1997
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APMIS
ISSN 0903-4641

Influence of aqueous extracts of medicinal plants on surface hydrophobicity of *Escherichia coli* strains of different origin

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Türi, M., Türi, E., Kõljalg, S. & Mikelsaar, M. Influence of aqueous extracts of medicinal plants on surface hydrophobicity of *Escherichia coli* strains of different origin. APMIS *105*: 956 962, 1997.

The adhesion of microbes on host cells is of decisive importance in the development of Gram-negative microbe-induced infections and can be influenced by the surface hydrophobicity of the microbial cell. The hydrophobicity of 155 *Escherichia coli* strains of different origin was determined by the salt aggregation test (SAT). Among the strains isolated from faecal samples of healthy persons only 16.7% showed aggregative properties, whereas among the strains isolated from the urine of patients with pyelonephritis and the faecal samples of calves and pigs with diarrhoea some 40.0%–60.0% were aggregative. The influence of aqueous extracts prepared from bearberry leaves, St. John's wort herbs, wild camomile and marigold flowers on hydrophobicity of 40 *E. coli* and 20 *Acinetobacter baumannii* strains was investigated. The decoctions of bearberry and St. John's wort increased remarkably the hydrophobicity of both microbial species. The infusions of wild camomile and marigold completely blocked the aggregative properties of the investigated strains. Bactericidal action was relatively low in the case of bearberry and St. John's wort and completely lacking in the case of wild camomile and marigold. Thus, one of the probable and potentially important action mechanisms of the four medicinal plants studied is their ability to influence the surface characteristics of the microbial cells and thereby their putative virulence properties.

Key words: *Escherichia coli*; medicinal plants; aqueous extracts; hydrophobicity.

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Escherichia coli is an opportunistic pathogen which normally colonizes the human intestinal tract. At the same time it is one of the most frequent etiological agents in urinary tract (UTI), wound and intestinal infections (19, 23, 24). In addition to antibacterial therapy, different alternative methods, particularly the application of aqueous extracts of medicinal plants, such as bearberry and wild camomile, have been used in the treatment of UTI and infectious diarrhoea (20). However, the mechanisms of action of medicinal plants have not yet been elucidated. One of the explanations might be their ability to intervene in the pathogenesis of infections caused by *E. coli*.

The most important virulence factor of microorganisms, in particular Gram-negative bacteria, is adhesion to host-cell receptors (25, 26). This adhesion is mediated by specific adhesins, which in Gram-negative bacteria are localized in fimbriae, e.g. pili (pilus adhesion), and in Gram-positive bacteria on the cell surface (nonpilus adhesion) (9). At the same time the bacteria can become associated with surfaces also by relatively nonspecific electrostatic or hydrophobic forces. Thus hydrophobicity of microorganisms serves as one of their adhesive

Received March 24, 1997.
Accepted July 1, 1997.

factors (9, 12). Some few data are available showing that the hydrophobicity of *E. coli* strains is variable, depending on the source of isolation of the particular strain (17).

The hydrophobic properties of the microbial cell wall can cause the aggregation of microorganisms (15, 16, 21). One possible way to study the aggregation capacity of different strains is using the salt aggregation test (SAT). SAT is technically easy to perform and possesses the necessary sensitivity (22). Using SAT as an experimental model, it may be possible to study the influence of different medicinal plants on the adhesive properties of clinical isolates of *E. coli*.

The aim of our work was to determine the hydrophobicity of *E. coli* strains of different origin and the influence on it of bearberry, St. John's wort, wild camomile and marigold, generally accepted medicinal plants of Europe.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The microorganisms used in this study were 155 strains of *E. coli* and 20 strains of *Acinetobacter baumannii*.

Out of 155 *E. coli* strains, 48 were isolated from faeces of healthy young (<20 years) persons: 30 collected during 1 year before the study and 18 recently isolated (Group 1); 30 from faeces of pigs and calves with infectious diarrhoea collected 2–3 years before the study; these strains were serotyped and they contained K 88 and/or K 99 antigen (Group 2); 55 from the urine of patients with pyelonephritis: 36 collected 1–3 years before the study and 19 recently isolated (Group 3); 21 from different clinical materials (blood, pus) collected during 1 year before the study and one collection strain *E. coli* K 12 (Group 4). Collection strain *E. coli* ATCC 25922 was used to elaborate the investigation methods. The cultures of *E. coli* were isolated from a single colony grown on McConkey agar plates and identified by conventional tube biochemical tests for *Enterobacteriaceae* (7).

To get comparative data, another Gram-negative opportunistic pathogen *A. baumannii* was tested with the same methods as for *E. coli*. *A. baumannii* strains originated from the lower respiratory tract and were selected from a previous point study as 10 strains with and without aggregative properties (14). The strains were identified as *A. baumannii* according to the following criteria: Gram-negative aerobic coccobacillus, oxidase negative, nonmotile and fermenting glucose. The following tests for biotyping were used: growth at 37° and 44°C, and utilization of single carbon sources such as L-phenylalanine, phenylacetate, levulinate, and citroconate, 4-hydroxybenzoate and L-tartrate (2).

Salt aggregation test (SAT)

The cultures of *E. coli* multiplied on agar slant at 37°C for 18–20 h. The microbial cells obtained from agar slant were suspended in 0.02 M potassium phosphate (Pp) buffer (pH 6.8) according to the 5 McFarland turbidity standard to a final concentration of 1.5×10^9 cells/ml. The *A. baumannii* strains were grown on blood agar for 24 h at 37°C. Colonies were suspended in 0.02 M Pp buffer (pH 6.8) for SAT.

SAT studies were performed using ammonium sulfate solutions (0.1, 0.5, 1.0, 1.5, 3.0 M) in 0.02 M Pp buffer (pH 6.8) as described by *Ljungh et al.* (1985). An aliquot (0.2 ml) of ammonium sulfate solution and additionally the same amount of Pp buffer for the control were pipetted into Nunclon® plate wells. To each well 0.2 ml of standardized microbial suspension was added so that the final concentrations for ammonium sulfate were 0.05, 0.25, 0.5, 0.75 and 1.5 M, and for microorganisms 0.75×10^9 cells/ml. The microtitre plates were gently rotated for 5 min. The presence of aggregation was examined by light microscopy (12.5×) using a dark background. The strains were tested for autoaggregation, using 0.02 M Pp buffer instead of ammonium sulfate.

SAT was defined as positive (+) if bacterial aggregation was clearly visible and negative (−) if no aggregation could be registered or if it was very weak. The SAT titre is defined as the lowest concentration of ammonium sulfate at which microbes still yield clearly visible aggregation. Strains autoaggregating in Pp buffer and/or expressing SAT titres 0.05 and 0.25 were considered highly aggregative/hydrophobic, and strains with titres 0.5–1.5 were considered low aggregative. A strain was considered nonaggregative if it did not express positive SAT even at a 1.5 M concentration of ammonium sulfate.

Plant materials and sample preparation

Some medicinal plants widely used in traditional European medicine were selected for study: the leaves of bearberry (*Arctostaphylos uva-ursi* (L.) Spreng. *Ericaceae*), the herb of St. John's wort (*Hypericum perforatum L. Guttiferae*), the flowers of wild camomile (*Matricaria recutita* L. *Asteraceae*) and marigold (*Calendula officinalis* L. *Asteraceae*). The quality of all the medicinal herbs met the requirements of pharmacopoeia (6). To prepare aqueous extracts, medicinal plants were reduced to powder with a diameter of the particles of less than 1 mm; 10 g of ground drugs was suffused with 100 ml of distilled water. The aqueous extracts of bearberry and St. John's wort were prepared as a decoction. For this purpose the decoction was boiled in a waterbath for 30 min and then immediately strained through cot-

ton. The aqueous extracts of wild camomile and marigold were prepared as infusions: the extracts were boiled in a waterbath for 15 min, and after cooling for 45 min they were strained. In both cases, after cooling the extracts were supplemented with distilled water up to 100 ml (10).

SAT assay with herbs

The influence of herb on SAT values of 40 clinical isolates of *E. coli* and 20 isolates of *A. baumannii* was studied. The 60 strains were selected according to their SAT titres: 30 strains expressing different SAT titres and 30 nonaggregative strains. The suspensions of microorganisms, 3×10^9 microbe/ml (10 McFarland), were prepared in 0.02 M Pp buffer. These suspensions were exposed for 15 min with the same amount of herb aqueous extracts to a final concentration of microbes 1.5×10^9. The SAT tests were then performed as described previously.

Testing of antibacterial activity of herbs

The antimicrobial activity, particularly the bactericidal effect, of aqueous extracts of herbs was tested against 20 strains of *E. coli*: 10 aggregative and 10 nonaggregative. The aqueous extracts of herbs were diluted in peptone broth 1:2, 1:4 and 1:8. The 0.05 ml aliquots of microbial suspensions in 0.02 M Pp buffer were seeded into undiluted aqueous extracts and into the prepared dilutions in tubes. The mixtures were incubated at 37°C for 18–20 h. After that, the bactericidal activity of herbs was determined by seeding different dilutions into broth after exposition, registering the absence or presence of overnight growth.

Statistics

Comparison of the distribution of SAT values in different groups was performed according to Kruskal-Wallis. The aggregation ability of *E. coli* strains was not dependent on time of preservation as determined by the χ^2 test.

RESULTS

Aggregation properties of E. coli

Out of 155 *E. coli* strains, 14 (9.2%) were autoaggregative or highly aggregative, 40 (25.7%) expressed aggregation in SAT at 0.5–1.5 M ammonium sulfate concentration, and the rest 101 (65.1%) were nonaggregative at a 1.5 M concentration of ammonium sulfate. The collection strain *E. coli* ATCC 25922 proved to be low aggregative (titre 1.5). It was found that the number of strains giving a positive SAT reaction increase in close association with the increase in the ammonium sulfate concentration. If a strain showed aggregation at a low concentration of ammonium sulfate, it also expressed a positive SAT at the last possible concentration (1.5 M) suitable for performing SAT.

To clarify whether the aggregative properties of *E. coli* strains were dependent on the length of storage of isolated cultures, we compared the aggregative properties of fresh and 1–3 year old isolates. Among the 37 freshly isolated strains 32.4% were aggregative, and among 118 strains isolated 1–3 years ago 35.6% were aggregative. The frequency of occurrence of aggregative and nonaggregative strains was similar in both groups ($p>0.05$).

Our experiments showed that the numbers of aggregative *E. coli* strains (titres up to 1.5) were different in the studied groups (Fig. 1). Thus, among the strains isolated from faeces of young healthy persons, only 16.7% of the strains (including 2.1% of autoaggregative ones) were aggregative, whereas among those isolated from calves and pigs with diarrhoea, 60.0% of the strains (including 13.3% autoaggregative) showed aggregation in SAT ($p<0.01$). There were also significantly more aggregative strains (40.0%) found in the urine of patients with pyelonephritis compared to faecal samples of healthy persons ($p\leq0.04$).

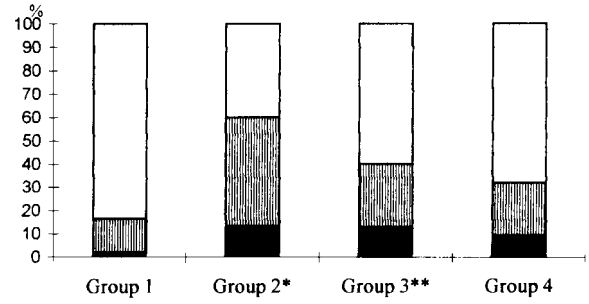

Fig. 1. Dependence of the prevalence of aggregative *Escherichia coli* strains on their origin.
* $p<00.1$ compared with values of Group 1.
** $p<0.04$ compared with values of Group 1.
■ autoaggregative+high aggregative strains ▥ low aggregative strains □ nonaggregative strains.
Group 1 – from faeces of healthy young (<20 years) persons: n=48. Group 2 – from faeces of pigs and calves with infectious diarrhoea: n=30. Group 3 – from urine of patients with pyelonephritis: n=55. Group 4 – from different clinical materials and collection strain *E. coli* K12: n=22.

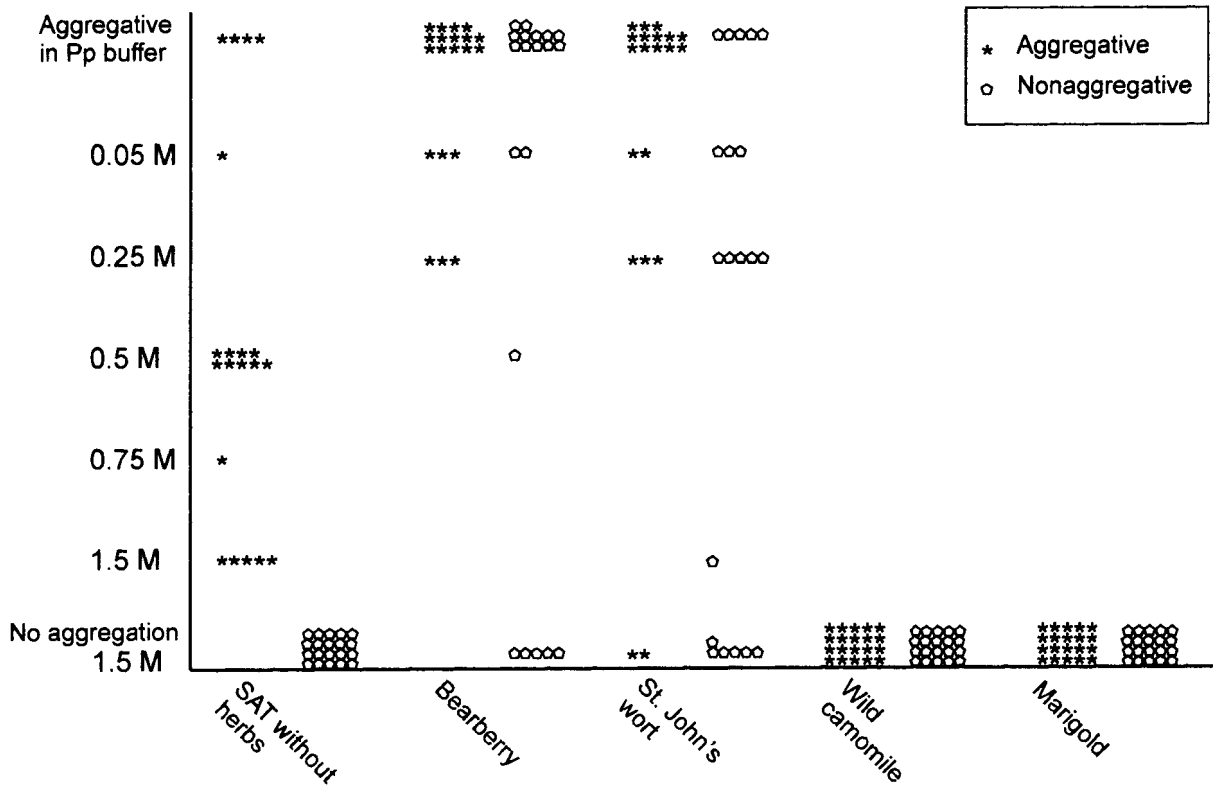

Fig. 2. SAT assays of aggregative (n=20) and nonaggregative (n=20) strains of *Escherichia coli* with herbs. The highest titres of SAT when aggregation was found or the strains proved to be nonaggregative.

Influence of different herbs on SAT

Different aqueous extracts of herbs showed a different influence on the SAT results. If in control experiments 15 *E. coli* strains showed low titres (0.5–1.5) of SAT (Fig. 2), then after exposure to bearberry and St. John's wort decoction their SAT titres increased 2–10 fold in 15 and 13 strains, respectively ($p<0.04$). Only two strains showed a decrease (no aggregation by 1.5 M ammonium sulfate) compared with their previous SAT titres.

A. baumannii aggregative strains used for exposition to herbs' aqueous extracts showed low titres (1.5) of SAT (Fig. 3). Similarly, an increase of SAT titres was universally registered after exposure to the bearberry decoction ($p<0.01$). Similar tendencies were not so clearly seen after exposure to St. John's wort decoction of *A. baumannii* strains. Likewise, more strains (four *A. baumannii versus* two *E. coli* strains) did not show any response to St. John's wort decoction.

In nonaggregative strains, the decoction of bearberry caused an increase in aggregation, as well as at high titres (0.25) both among *E. coli* and *A. baumannii* strains (Figs. 2 & 3). After exposure to St. John's wort decoction, the SAT response was not so universally homogeneous.

The infusions of wild camomile and marigold blocked SAT both in aggregative *E. coli* and *A. baumannii* strains, and did not exert any influence on the SAT results of nonaggregative strains (Figs. 2 & 3).

Antimicrobial activity of aqueous extracts of bearberry, St. John's wort, wild camomile and marigold

The antimicrobial activity of the investigated herbs was low (Table 1). The leaves of bearberry possessed the highest antimicrobial activity. There was no growth registered after the exposure of 20 different *E. coli* strains to the undiluted decoction of bearberry and in the case of two-fold dilution only one strain showed growth afterwards in peptone broth.

Some antimicrobial activity of undiluted de-

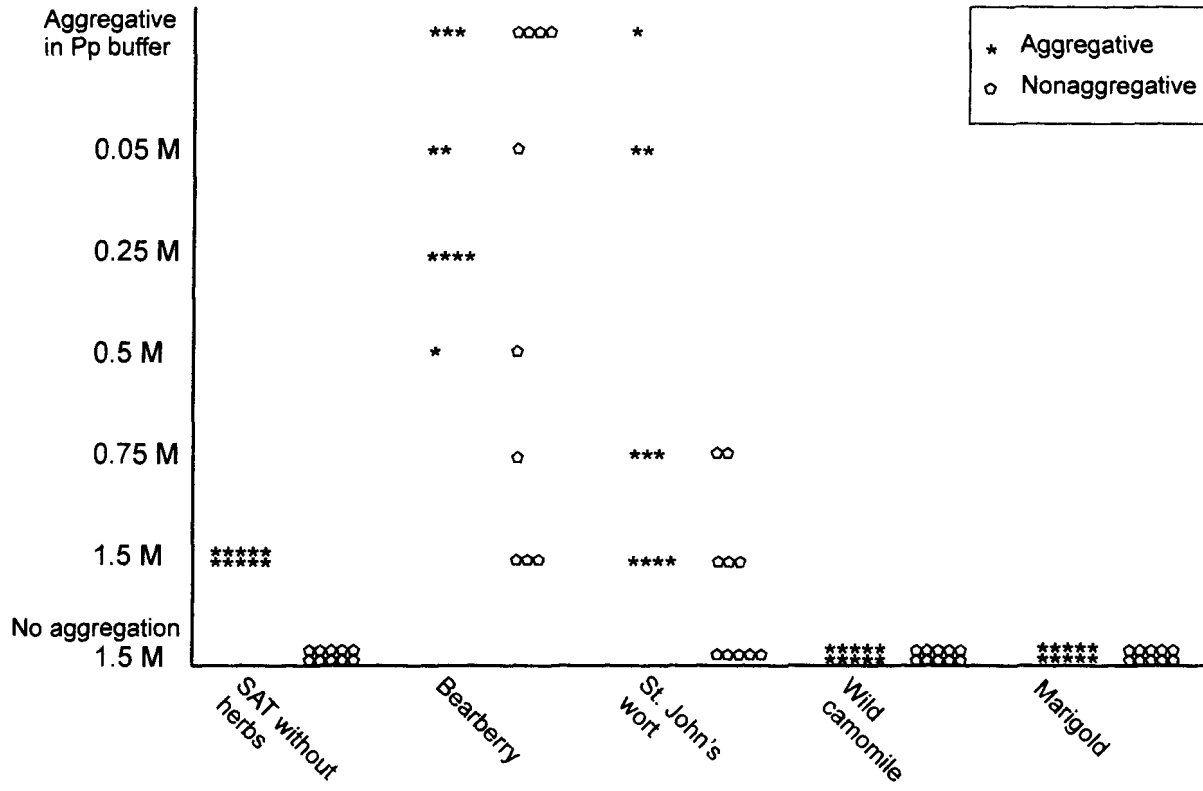

Fig. 3. SAT assays of aggregative (n=10) and nonaggregative (n=10) strains of *Acinetobacter baumannii* with herbs. The highest titres of SAT when aggregation was found or the strains proved to be nonaggregative.

coction of St. John's wort could also be found as only 3 of 20 strains showed growth in peptone broth after their exposure to the herb. However, a diluted decoction of St. John's wort did not kill any *E. coli* strain.

The infusions of wild camomile and marigold did not show any antimicrobial activity against the 20 tested *E. coli* strains.

DISCUSSION

We found that the hydrophobic properties of different *E. coli* strains varied depending on their source of isolation. The hydrophobicity of the investigated *E. coli* strains could be modulated by aqueous extracts of some investigated medicinal plants: the decoctions of bearberry and St. John's wort significantly enhanced their hydrophobicity, while wild camomile and marigold blocked it totally. These seem to be the first experiments proving that medicinal plants can change the surface properties of Gram-negative bacteria.

The hydrophobic properties of *E. coli* strains were studied by the salt aggregation test: at different concentrations of ammonium sulfate, the strains of Gram-negative microorganisms showed different capacity for aggregation.

TABLE 1. *Resistance of* Escherichia coli *strains (n=20) to the bactericidal activity of aqueous extracts of medicinal plants*

Medicinal plants	Aqueous extracts	Number of strains showing growth after exposure to aqueous extracts			
		non diluted	1:2	1:4	1:8
Bearberry	Decoction	0	1	15	19
St. John's wort	Decoction	3	20	20	20
Wild camomile	Infusion	20	20	20	20
Marigold	Infusion	20	20	20	20

Among the 155 *E. coli* strains tested, about one third (34.9%) were hydrophobic, whilst two-thirds (65.1%) were nonhydrophobic. It has been shown by *Ljungh et al.* (1985) that also among staphylococci and streptococci isolated from patients with wound infections both hydrophobic and nonhydrophobic strains occurred. Thus, our results and data in the literature confirm the suitability of SAT for the determination of differences in hydrophobic cell wall properties of *E. coli* (21).

We managed to show that the aggregative capacity of particular strains determined by SAT was a nonoccasional marker. First, it was found that the number of strains, giving a positive SAT reaction increased in close association with the increase in ammonium sulfate concentration. Secondly, we showed that storage of *E. coli* strains does not change their hydrophobicity.

The aggregative capacity was dependent on the origin of the isolated *E. coli* strains. At the highest possible concentration (1.5 M) of ammonium sulfate, the strains of *E. coli* from the urine of patients with pyelonephritis and the strains from animals with diarrhoea showed aggregative properties in 40.0% and 60.0% cases, respectively. At the same concentration, among the strains isolated from faeces of healthy persons, only 16.7% strains showed aggregation. So, for a pathogenic process to start, a microorganism has to stick either to mucous membranes or mucosa (11). Thus, it can be supposed that an increased hydrophobicity serves in particular strains of some Gram-negative bacteria as a putative virulence marker.

Medicinal plants have mainly been applied to alleviate inflammatory processes in urinary tract and wound infections, and also in intestinal disorders and several other diseases. When studying their effect, investigators have often restricted themselves to the antimicrobial properties of medicinal plants (1, 13). In this case only the aqueous extract of bearberry leaves, and to a lesser extent that of St. John's wort, showed a moderate antimicrobial activity against tested *E. coli* strains. Thus, the antimicrobial activity of all tested medicinal plants cannot be considered the only mechanism of their putative action in the treatment of microorganism-induced infections (5)

It is hard to predict which compounds of medicinal plants might interfere with the cell-surface hydrophobicity of *E. coli* and *A. baumannii.* The studied medicinal plants have been shown to contain multiple biologically active compounds (3). For example, in the ethereal oil of wild camomile over 30 different components have been determined. In addition, the plant contains 19 flavonoids, seven amino acids and some mucinous compounds. Out of the many different compounds of bearberry and St. Johns' wort decoctions which showed similar aggregation-enhancing effects on SAT of *E. coli* and *A. baumannii* we could trace large amounts of some tannins in both (3). In contrast, the herbs which blocked the aggregation of these Gram-negative bacteria in a similar manner contain considerably smaller amounts of tannins, while mucinous substances could be found in both of them.

The capability of aqueous extracts of medicinal plants to enhance or lower the hydrophobicity of Gram-negative bacteria *in vitro* allows the suggestion that the same mechanism also influences microbial adhesion *in vivo.* The total blocking of bacterial hydrophobicity *in vitro* by aqueous extracts of wild camomile and marigold could explain their widespreed use in traditional medicine. Lately it has been shown that preparations that reduce the hydrophobicity of microorganisms (e.g. Sorbact 10^5) are effective in the treatment of staphylococcus- and streptococcus-induced wound infections (8).

The ability of the aqueous extract of bearberry to enhance the hydrophobicity and aggregation of Gram-negative bacteria suggests that in the case of urinary tract infections microbial particles might be more easily aggregated and excreted *per vias naturales* (4).

Further studies are in progress to determine more precisely the aggregation-blocking or -enhancing compounds of medicinal plants in order to apply their properties in the production of new bioactive medicines.

This investigation was supported by a grant from the *Estonian Science Foundation.*

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