Biocatalysis and Agricultural Biotechnology 21 (2019) 101328

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

Efficacy of crude extracts of *Clitoria ternatea* for antibacterial activity against gram negative bacterium (*Proteus mirabilis*)

S. Dhanasekaran [a], A. Rajesh [a], Thangavel Mathimani [b], S. Melvin Samuel [c], Rajasree Shanmuganathan [d], Kathirvel Brindhadevi [e,*]

[a] Department of Biotechnology, Thanthai Hans Roever Arts and Science College, Perambalur, 621 212, Tamil Nadu, India
[b] Department of Energy and Environment, National Institute of Technology, Tiruchirappalli, 620 015, Tamil Nadu, India
[c] Department of Materials Science and Engineering, CEAS, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53211, United States
[d] Institute of Research and Development, Duy Tan University, Da Nang, 550000, Viet Nam
[e] Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

ARTICLE INFO

Keywords:
Urinary tract infection
Proteus mirabilis
Clitoria ternatea
Bioactive compounds
Plant extract
Antibacterial activity

ABSTRACT

In the present study, antimicrobial activities of the crude extracts of the plant *Clitoria ternatea* were tested against the urinary tract infection causing pathogen *Proteus mirabilis.* Initially, 63 clinical samples from different age groups were collected for evaluating the urinary tract infection. Then, the clinical samples were examined for the presence of *Proteus mirabilis* bacterium by assessing morphological, biochemical, and cultural characteristics. To assess the zones of inhibition for various antibiotics towards *Proteus mirabilis*, disc diffusion method involving standard antibiotic discs was used. The antibacterial activities of antibiotics against *Proteus mirabilis* showed that ciprofloxacin, gentamycin and tetracycline exhibited better zones of inhibition. Further, the extracts from the leaves of *Clitoria ternatea* were prepared with different solvents such as acetone, isopropyl alcohol and petroleum ether and the extract yield was higher in acetone than the other two solvents. The highest antibacterial activity was observed for acetone and the lowest antibacterial activities were observed for isopropyl alcohol, and petroleum ether extracts against *Proteus mirabilis.* In addition, phytochemical analysis revealed the presence of several bioactive compounds.

1. Introduction

Urinary Tract Infection (UTI) is a very common and harmful bacterial infection, which affects several individuals every year. It can be managed either by visiting the physicians or by hospitalization (Masajtis-Zagajewska and Nowicki, 2017). Presence of microorganisms in the sterile urinary tract is identified to be UTI. UTI relies on age, gender, and also other factors and UTI occurrence is higher in the 1st year of childhood and then, will reduce markedly after infancy among boys (Jakobsson et al., 1999; Zorc et al., 2005). Further, maximum UTI has been identified among women relatively to men; this is owing to the adherence of urothelial mucosa to micro polysaccharide or anatomical predisposition (Schaeffer et al., 2001). Bacterial pathogens namely *Proteus, Enterobacter, Klebsiella, Pseudomonas, Serratia* sp., *Escherichia coli*, *Enterococcus* sp., and *Staphylococcus aureus* are the most commonly found in UTI (Zorc et al., 2005). On contrary, the nonbacterial mode of UTI consists of hemorrhagic cystitis and *Candida* infections (Schaeffer et al., 2001).

Proteus mirabilis, a gram negative bacterium is the very common causative agent of UTI (Ehsan et al., 2018; Mobley and Belas, 1995; Park et al., 2019; Torzewska et al., 2019). Further, extended spectrum beta-lactamase producing bacteria have been identified among the members of *Enterobacteriaceae,* which also cause UTI both in and outpatients (Mendelson et al., 2005). However, several antimicrobial regimens containing a variety of drugs, doses, and durations are being used to cure UTIs (Warren et al., 1999). In a perspective of treating UTIs, bacterial resistance towards various antibiotics has paved the way to employ expensive broad spectrum drugs (Pugazhendhi et al., 2017; Soulsby, 2005). Therefore, there is a crucial need of research attempts to explore potential and competent antibacterial components against UTI causing pathogens. Bioactive compounds extracted from plants and algae showed antibacterial activity against *Proteus mirabilis.* In the case

* Corresponding author.
E-mail address: kathirvelbrindhdevi@tdtu.edu.vn (K. Brindhadevi).

https://doi.org/10.1016/j.bcab.2019.101328
Received 18 August 2019; Received in revised form 27 August 2019; Accepted 31 August 2019
Available online 1 September 2019

of algal bioactive compounds, algae produce various bioactive compounds having antibacterial activity due to their diversified physiological characteristics (Pugazhendhi et al., 2018; Sudhakar et al., 2019). It is reported that bioactive compounds extracted from algae revealed anti-bacterial activity against *P. mirabilis* (Villarreal-Gómez et al., 2010). In concern with plant sources for bio-active compounds, numerous medicinal plants are being examined as they have higher active biomolecule contents, which can be used as a therapy for any hazard. The natural materials were used as antimicrobial agents over the long years (Balasundar, 2011; Saravanan et al., 2018).

Bioactive components and countless important drugs have been extracted from medicinal plants and antibacterial activities of lots of plant extracts have been described so far (Pandey and Mishra, 2010). Medicinal plants exhibit extensive pharmacological properties such as antipyretic, antimicrobial, anti-inflammatory, anticancer, and antioxidant, etc. (Al-Snafi, 2015, 2016a). Among the plants, *Clitoria ternatea* Linn belongs to the fabaceae family predominantly present in the tropical and subtropical areas and is the most generally exploited medicine in India. However, antimicrobial activity of the biologically active compounds extracted from *Clitoria ternatea* need to be assessed against suitable UTI causing pathogens. Further, the minimum inhibitory concentration of the plant extracts needs to be identified to confirm its potential activity. Eventually, optimal parameters can be studied to augment its activity for UTI therapy. Keeping these concerns, this present study was undertaken with the following objectives; (i) Purification and morphological identification of *Proteus mirabilis* from clinical samples, (ii) Evaluation of antibacterial activity of various antibiotics towards *Proteus mirabilis* (iii) extraction of bioactive compounds from *Clitoria ternatea Linn.* Using acetone, isopropyl alcohol, and petroleum ether solvents, (iv) Determination of antibacterial activity of the three crude extracts against *Proteus mirabilis* through disc diffusion method.

2. Material and methods

2.1. Collection of samples and identification of Proteus mirabilis

Totally 63 clinical samples from different age groups were collected for evaluating the urinary tract infection and the clinical samples were promptly brought to the laboratory aseptically for further processing. The isolated clinical samples were serially diluted and were streak plated on various selective media, namely cysteine lactose electrolyte deficient agar, UTI nutrient agar, MacConkey agar medium, nutrient agar and incubated at 37 ± 1 °C for about 24 h. After incubation, the plates were evaluated for biochemical and morphological characteristics.

2.2. Antibacterial activity of the selected antibiotics against Proteus mirabilis

Muller hinton agar medium was used to study the antibacterial activity of the chosen antibiotics by disc diffusion technique. Antibiotics such as amikacin, ampicilin, amoxyclav, amoxicillin, cloxacillin, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, gentamycin, ofloxacin, rifampicin, tetracycline, and vancomycin were used in this study. The inoculation of all the agar plates with *Proteus mirabilis* isolate was taken place as mentioned below; sterile cotton plunged in *Proteus mirabilis* inoculum was swabbed on the selected agar plates and then, the chosen antibiotics mentioned above were placed on the plates and incubated as above (Boudjemaa et al., 2019; Howery et al., 2018). Afterwards, the antibacterial activity of each antibiotic in terms of zone of inhibition was measured.

2.3. Collection of plant material and extraction of plant metabolites

Collated leaves of *Clitoria ternatea* were washed with running tap water followed by distilled water wash. Leaves of *Clitoria ternatea* were shade dried for about 5–7 days and then, placed in a hot air oven at 55 °C for 5 h to get rid of any moisture. The dried plants were ground into soft powder and loaded in a clean dry soxhlet apparatus. The solvents used were acetone, isopropyl alcohol and petroleum ether and refluxed for about 2–3 h to obtain desirable plant extracts using different solvents. Then, the different solvent extracts were evaporated to obtain solvent free extracts. The quantity of each extract was calculated gravimetrically.

2.4. Antimicrobial activity of plant extracts

As mentioned earlier, Muller hinton agar medium was used to study the antibacterial activities of different solvent extracts through disc diffusion technique. For experimentation, 100, 200, 300, 400 and 500 µg of each crude extract were loaded on the discs. The discs loaded with *Clitoria ternatea* extracts were kept on the agar plates swabbed with *Proteus mirabilis* and allowed for incubation as above. Afterwards, the antibacterial activities of the extracts in terms of zones of inhibition were measured.

3. Results and discussion

3.1. Sample collection and identification

In this study, totally 63 clinical samples were collected and they were further analyzed for the presence of the UTI causing pathogen, *Proteus mirabilis.* From the collected samples, 47 samples were positive and remaining 16 samples were negative. The positive and negative result of the collected samples is given in Table 1. Among the positives, 27 samples were positive exclusively for *Proteus mirabilis* bacterium whereas 20 samples also showed the presence of other UTI causing pathogens. The identification of *Proteus mirabilis* was done based on the morphological features of the colonies grown on different media.

3.2. Antibacterial activity of antibiotics against Proteus mirabilis

Antibacterial activities of the chosen antibiotics against *Proteus mirabilis* were determined through disc diffusion technique. The zone of inhibition developed by each antibiotic against *Proteus mirabilis* has been portrayed in Fig. 1.Ciprofloxacin, gentamycin and tetracycline displayed the higher zones of inhibition against *Proteus mirabilis* and the measured zones of inhibition were between 19-20 mM.

On contrary, lower zones of inhibition was observed for cloxacilin, and ampicilln antibiotics, which indicated the low antibacterial activities of the antibiotics against *Proteus mirabilis*. From Fig. 1, it is clearly seen that cloxacilin, and ampicillin revealed minimum antibacterial activities against *P. mirabilis* in the range between 11-12 mM. From this study, it was concluded that the growth of *Proteus mirabilis* was reduced by the ciprofloxacin, gentamycin and tetracycline antibiotics. Similar works on antibacterial activity were done by various researchers. Nalixidic acid showed a high inhibitory action against *Proteus mirabilis* using disc diffusion method (Barlow, 1963; Lesher et al., 1962). In other report, amoxycillin and cotrimoxazole were found to show higher zones of inhibition against *Proteus mirabilis* (Astal et al., 2002). Another study has proposed that ofloxacin and perfloxacin can be considered for the

Table 1
Collection of urine samples.

S.No	Age Range of the Patients (in year)	Total No. of samples	
		Male	Female
1.	21–30	11	12
2.	31–40	7	16
3.	41–50	6	6
4.	51–60	2	3
Total no. of samples		**26**	**37**

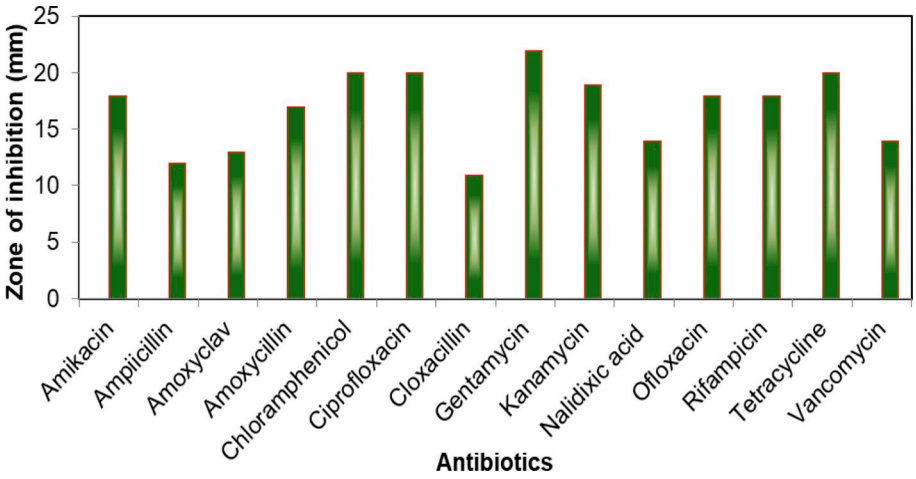

Fig. 1. Antibacterial activity of the selected antibiotics against *Proteus mirabilis*.

treatment of *Proteus mirabilis* infections (Momoh et al., 2007).

3.3. Antibacterial activity of plant extracts

The time taken for extracting the bioactive compounds differs based on the plant material and the type of solvents used. The percentages of extracts obtained from *Clitoroia ternatea* using different solvents is illustrated in Fig. 2. The higher extract content was noticed while using acetone solvent, which was 16.2%. Isopropyl alcohol and petroleum ether showed about 12.2% and 9.7% extract contents, respectively. Further, in order to evaluate the antibacterial activities of the extracts, concentrations of the extract in the range between 100 - 500 µg were used against *Proteus mirabilis*. Antibacterial activities (in terms of zones of inhibition) of the different solvent extracts of *Clitoria ternatea* against *Proteus mirabilis* are presented in Fig. 3. The highest zone of inhibition was observed for the acetone extract of *Clitoroia ternatea* and lower inhibitory effects were observed for the isopropanol and petroleum ether extracts of *Clitoria ternatea*. From Fig. 3, it is apparent that the lowest concentration of the extract showed minimum antibacterial activity against *Proteus mirabilis*, irrespective of the solvent used. In the present study, acetone, isopropanol and petroleum ether were used as extraction solvents for obtaining the crude extracts from *Clitoria ternatea leaves*. Acetone extract of *Clitoria ternatea* showed antimicrobial activity within the range of 20–26 mM against *Proteus mirabilis*. It has been previously reported in literature that the solvent extracts of *Clitoria ternatea* were found to have antimicrobial activities against UTI causing pathogens (Uma et al., 2009).

In the case of concentration of extract, a report stated that the

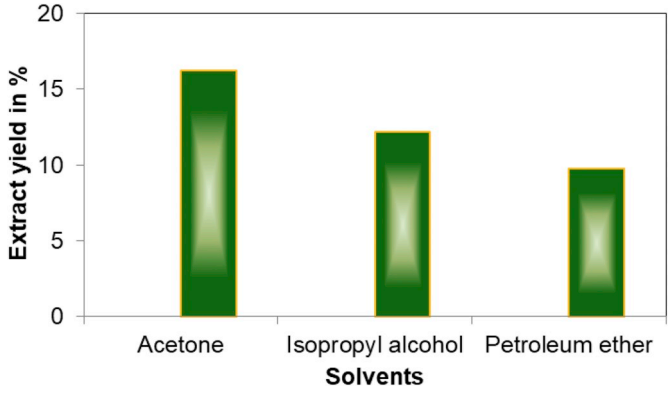

Fig. 2. Extract yield of *Clitoria ternatea* Linn. using acetone, isopropyl alcohol and petroleum ether.

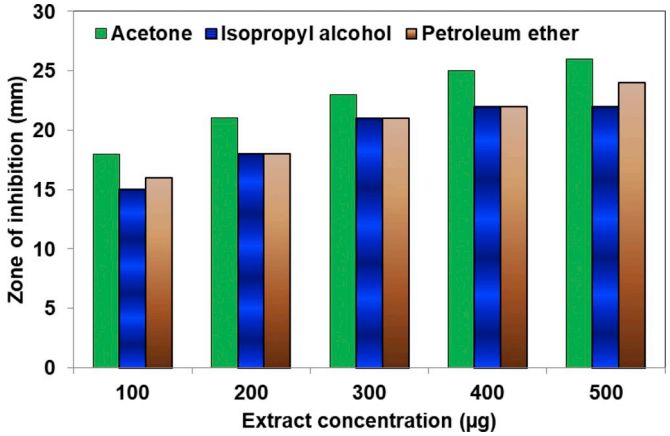

Fig. 3. Extract yield of *Clitoria ternatea* Linn. using acetone, isopropyl alcohol and petroleum ether.

ethanolic extracts have shown maximal antibacterial activities against *Proteus mirabilis* (Arora and Kaur, 2007). Another similar work has indicated that the methanolic extract of *Clitoria ternatea* was tested for its antimicrobial activity against 11 UTI causing pathogens (Balasundar, 2011). As the *Clitoria ternatea* extracts revealed better antibacterial activities, it would be significant to ascertain the components present in the extract. It is stated that *Clitoria ternatea* is a potential medicinal plant for various medical uses as it has broad range of pharmacological activities (Al-Snafi, 2016b).

Preliminary phytochemical analysis of the extract showed the presence and absence of various bioactive compounds as mentioned below. Compounds found in the acetone extract were alkaloids, protein etc., whereas no anthraquinone glycosides or flavonoids were present. Similarly, in isopropyl alcohol extract, proteins, amino acids, and carbohydrates, were present while alkaloids, tannins and phenolics were absent. On contrary, alkaloids, carbohydrates, tannins and phenolics were observed in the petroleum ether extract while anthraquinone glycosides, flavonoids, saponins, and phytosterol were not present. Alkaloids, tannins, phenolics flavonoids, and terpenoids are the key bioactive compounds with various pharmacological activities (Sudhakar et al., 2019). These data were in good agreement with the other reports. In a previous research work, extracts of *Clitoria ternatea* were found to contain protein, carbohydrate, alkaloids, saponins flavonoids, and tannins (Uma et al., 2009). In another study, the extract obtained from *Clitoria ternatea* was found to contain tannins, taraxerol, starch, ternatins and resins (Terahara et al., 1996).

4. Conclusion

In this study, screening, purification and identification of *Proteus mirabilis* from the clinical samples were performed and then, antibacterial activities of the selected antibiotics were tested. The antibacterial activity testing of antibiotics against *Proteus mirabilis* showed that ciprofloxacin, gentamycin and tetracycline exhibited better zones of inhibition. Further, the extracts from the leaves of *Clitoria ternatea* were obtained using acetone, isopropanol, and petroleum ether solvents. Extracts obtained from *Clitoria ternatea* with acetone, isopropanol, and petroleum ether were evaluated for antibacterial activities against *Proteus mirabilis* and the highest activity was observed for the cetone extract. Hence, it was concluded that *Clitoria ternatea* would be a potential medicinal plant for treating UTI and however, the bioactive compounds need to be purified and quantified to ascertain the appropriate compound, which plays a crucial role in conferring antimicrobial activity of *Clitoria ternatea* against *Proteus mirabilis*.

References

Al-Snafi, A.E., 2015. Chemical constituents and pharmacological importance of Agropyron repens–A review. Res. J. Pharm. Technol. 1, 37–41.

Al-Snafi, A.E., 2016. Chemical constituents and pharmacological effects of Clerodendrum inerme-A review. SMU Med. J. 3, 129–153.

Al-Snafi, A.E., 2016. Pharmacological importance of Clitoria ternatea–A review. IOSR J. Pharm. 6, 68–83.

Arora, D.S., Kaur, G.J., 2007. Antibacterial activity of some Indian medicinal plants. J. Nat. Med. 61, 313–317.

Astal, Z.Y., Sharif, F.A., Abdallah, S.A., Fahd, M.I., 2002. Multiresistant Escherichia coli isolated from women with community-acquired urinary tract infections in the Gaza Strip. J. Chemother. 14.

Balasundar, A., 2011. Antimicrobial activity of the leaf extracts of two medicinal plants against MRSA (Methicilin Resistant Staphylococcus aureus) from human urinary tract pathogens. Res. J. Microbiol. 6, 625–631.

Barlow, A., 1963. Nalidixic acid in infections of urinary tract. Br. Med. J. 2, 1308.

Boudjemaa, H., Allem, R., Zouagui, S., el Houda Khennouchi, N.C., Kerkoud, M., Rolain, J.M., 2019. Molecular drivers of emerging multidrug resistance in Proteus mirabilis clinical isolates from Algeria. J. Glob. Antimicrob. Resist. 18, 249–256. https://doi.org/10.1016/j.jgar.2019.01.030.

Ehsan, N., Ahmad, S., Navid, A., Azam, S.S., 2018. Identification of potential antibiotic targets in the proteome of multi-drug resistant Proteus mirabilis. Meta Gene 18, 167–173.

Howery, K.E., Şimşek, E., Kim, M., Rather, P.N., 2018. Positive autoregulation of the flhDC operon in Proteus mirabilis. Res. Microbiol. 169, 199–204.

Jakobsson, B., Jacobson, S., Hjälmås, K., 1999. Vesico-ureteric reflux and other risk factors for renal damage: identification of high-and low-risk children. Acta Paediatr. 88, 31–39.

Lesher, G.Y., Froelich, E.J., Gruett, M.D., Bailey, J.H., Brundage, R.P., 1962. 1, 8-Naphthyridine derivatives. A new class of chemotherapeutic agents. J. Med. Chem. 5, 1063–1065.

Masajtis-Zagajewska, A., Nowicki, M., 2017. New markers of urinary tract infection. Clin. Chim. Acta 471, 286–291.

Mendelson, G., Hait, V., Ben-Israel, J., Gronich, D., Granot, E., Raz, R., 2005. Prevalence and risk factors of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in an Israeli long-term care facility. Eur. J. Clin. Microbiol. Infect. Dis. 24, 17–22.

Mobley, H.L., Belas, R., 1995. Swarming and pathogenicity of Proteus mirabilis in the urinary tract. Trends Microbiol. 3, 280–284.

Momoh, A., Odike, M., Olowo, S., Momoh, A., Okolo, P., 2007. Resistance pattern of urinary tract infection bacterial isolates to selected quinolones. Benin J. Postgrad. Med. 9.

Pandey, R., Mishra, A., 2010. Antibacterial activities of crude extract of Aloe barbadensis to clinically isolated bacterial pathogens. Appl. Biochem. Biotechnol. 160, 1356–1361.

Park, S.Y., Yu, S.N., Lee, E.J., Kim, T., Jeon, M.H., Choo, E.J., Park, S., Chae, J.W., Bang, H.I., Kim, T.H., 2019. Monomicrobial gram-negative necrotizing fasciitis: an uncommon but fatal syndrome. Diagn. Microbiol. Infect. Dis. 94, 183–187.

Pugazhendhi, A., Dhanarani, S., Shankar, C., Prakash, P., Ranganathan, K., Saratale, R. G., Thamaraiselvi, K.J.M.p., 2017. Electrophoretic pattern of glutathione S-transferase (GST) in antibiotic resistance Gram-positive bacteria from poultry litter, 110, 285–290.

Pugazhendhi, A., Prabakar, D., Jacob, J.M., Karuppusamy, I., Saratale, R.G.J.M.p., 2018. Synthesis and characterization of silver nanoparticles using Gelidium amansii and its antimicrobial property against various pathogenic bacteria, 114, 41–45.

Saravanan, M., Senthilkumar, P., Kalimuthu, K., Chinnadurai, V., Vasantharaj, S., Pugazhendhi, A.J.I.c., products, 2018. Phytochemical and pharmacological profiling of Turnera subulata Sm., a vital medicinal herb. Ind. Crops Prod. 124, 822–833.

Schaeffer, A.J., Rajan, N., Cao, Q., Anderson, B., Pruden, D.L., Sensibar, J., Duncan, J., 2001. Host pathogenesis in urinary tract infections. Int. J. Antimicrob. Agents 17, 245–251.

Soulsby, E.J., 2005. Resistance to Antimicrobials in Humans and Animals. British Medical Journal Publishing Group.

Sudhakar, M., Kumar, B.R., Mathimani, T., Arunkumar, K., 2019. A review on bioenergy and bioactive compounds from microalgae and macroalgae-sustainable energy perspective. J. Clean. Prod. 228, 1320–1333.

Terahara, N., Oda, M., Matsui, T., Osajima, Y., Saito, N., Toki, K., Honda, T., 1996. Five new anthocyanins, ternatins A3, B4, B3, B2, and D2, from Clitoria ternatea flowers. J. Nat. Prod. 59, 139–144.

Torzewska, A., Bednarska, K., Różalski, A., 2019. Influence of various uropathogens on crystallization of urine mineral components caused by Proteus mirabilis. Res. Microbiol. 170, 80–85.

Uma, B., Prabhakar, K., Rajendran, S., 2009. Phytochemical analysis and antimicrobial activity of Clitorea ternatea Linn against extended spectrum beta lactamase producing enteric and urinary pathogens. Asian J. Pharmaceut. Clin. Res. 2, 94–96.

Villarreal-Gómez, L.J., Soria-Mercado, I.E., Guerra-Rivas, G., Ayala-Sánchez, N.E., 2010. Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface, 45, 267–275.

Warren, J.W., Abrutyn, E., Hebel, J.R., Johnson, J.R., Schaeffer, A.J., Stamm, W.E., 1999. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Clin. Infect. Dis. 29, 745–759.

Zorc, J.J., Kiddoo, D.A., Shaw, K.N., 2005. Diagnosis and management of pediatric urinary tract infections. Clin. Microbiol. Rev. 18, 417–422.