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Original Article

Anti-urinary tract infection activity of selected herbal extract towards isolated *Kosakonia cowanii* (OQ 073698)

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ABSTRACT

Urinary Tract Infection (UTI) is one of the most common types of infection caused by the gastrointestinal tract of humans. UTI is the most common in women because a women's urethra is shorter. In the present investigation, four UTI-causing bacteria were isolated on MacConkeys as well as on UTI agar media which is selective for UTI pathogens only. The isolated bacteria were tested for antibiotic resistance towards the selective antibiotics Ampicillin (10µg), Penicillin (10µg), and Chloramphenicol (30µg). Based on antibiotic resistance and morbific nature, the organism was screened. On the basis of morphological, biochemical characteristics and the 16S r-RNA sequencing method the organism was identified as *Kosakonia cowanii.* The nucleotide sequence was deposited to NCBI and received a unique accession number (OQ 073698). The anti-UTI activity was performed towards selective medicinal herbs by using ethanol, methanol and water as solvent extraction methods. The *Embilica officinalis* ethanolic extract (1 mg/ml) and standard antibiotic chloramphenicol showed 1.8 mm zone of inhibition against *K. cowanii* (OQ 073698). From the present study, it is concluded that *E. officinalis* ethanolic extract was effective to treat UTI infection. Another core finding from the present study includes - an isolated pathogen that was earlier resistant to ampicillin but when combines with *E. officinalis* and *Boerhavia diffusa* ethanolic extracts separately showed 2.0 mm zone of inhibition.

1. Introduction

Urinary Tract Infections (UTIs) are one of the most common bacterial infections, with a frequency 50-60% in mature females [1]. Additionally, this infection is associated with substantial healthcare and societal costs which are only in the United States of America (USA); UTIs are responsible for 7 million clinic visits annually [2]. Except among infants, the infection occurs more commonly in women than in men and it was estimated that about 40–50% of women experience one time at least in their lives and 20–30% of them have other episodes [3]. Urinary tract infections could be uncomplicated or complicated. Uncomplicated UTIs could be separated into an infection of lower UTIs (cystitis) and upper UTIs (Pyelonephritis) [4].

Fundamental factors responsible for cystitis are gender, former UTI infection, genetic susceptibility, vaginal infection, sexual activeness, overweight and diabetes too [5]. UTI is mostly caused by bacteria, through other microorganisms such as fungi or viruses which are rare etiological agents [6]. Among the uropathogens, *Escherichia coli* is the most common bacteria (75–90% of isolates) in both community and hospital infections, whereas other pathogenic bacteria like *Proteus mirabilis*, *Staphylococcus saprophyticus*(with

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particularly frequent isolation from younger female), *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are less occurs [2, 3, 7]. The uropathogenic bacteria express fimbrial adhesions to glycolipids and glycoproteins on the epithelial surface of the cell and by these mechanisms bacteria can overcome the flow of urine and maintain in the urinary tract. The bacteria also produce other substances like toxins, hemolysin, and colony-necrotizing factors; can disrupt the epithelial integrity, permit bacterial invasion, and, therefore, enhance the risk of infection also. Uropathogens can internalize into host epithelial cells and divide inside there so that it provides a reservoir for recurrent infection [7, 8]. In most cases, these uropathogens begin to colonize the surface of the perineum and periurethral and lead to the development of infection. Colonization of these bacteria could be inhibited by the normal microbiota of humans, such as *Staphylococcus epidermidis*, *Lactobacillus* spp, and *Corynebacteria* [9]. Additionally, bacterial colonization and preliminary infection can be eliminated by host defense mechanisms in the bladder.

Considering the severity of the infection, several treatments are applied to treat UTI infections however, extensive use of antibiotics leads to the emergence of antibiotic resistance in microbes. To solve the antibiotic resistance problem, Complementary and Alternative Medicine (CAM) has been recognized as an effective approach [10-13]. CAM consists of a wide range of products such as natural compounds, dietary supplements such as vitamins and minerals and probiotics [14]. Clinical research also suggests, the best natural option for long-term prevention of UTI as the intake of probiotics, medical herbs and vitamins [15-17]. Considering the UTI severity and antibiotics resistance problem; CAM could provide desirable results, especially when combined with a routine antibiotic regimen. With the same hypothesis, present investigation an attempt has been made to isolate the UTI pathogens and exploration of potential herbal extracts to treat UTI was studied.

2. Materials and Methods

2.1. Sample collection

The urine samples from UTI-causing patients with their ethical consent were collected from the Government Medical Hospital Nanded in sterile containers and transported to the laboratory and stored in the refrigerator separately for further study.

2.2. Isolation of UTI-causing pathogens

A loopful of well-mixed urine samples was spread on MacConkey's agar plates. All the plates were incubated at 37℃ for 24 h. Further, the well-isolated bacterial colonies with different morphologies were selected. Pure cultured colonies of the isolated bacteria were further streaked on UTI agar plate. The UTI agar plates were incubated at 37℃ for 24 h. The well-isolated colonies were further stored on to slants for further study [18].

2.3. Screening and Identification of Antibiotic-Resistant Uropathogen

The screenings of the uropathogenic were subjected to access antibiotic resistance activity on the Muller Hinton agar plate by disc diffusion method. Antibiotic resistance patterns of all selected isolates were determined on Muller Hinton agar plates by the Kirby-Bauer disc diffusion method. The selected screened isolates were declared as sensitive or resistant on the basis of the zone of inhibition, following the criteria of the Clinical Laboratory Standards Institute [19]. The selected antibiotics in the present investigation were Penicillin (10ug), Ampicillin (10ug), and Chloramphenicol (30ug). The isolates had resistance to all the antibiotics under study from the selected bacteria and were carried forward for further study. The selectively screened isolates were tested secondarily for the optical density after 24 h. One of the bacteria having antibiotic resistance and observed the highest optical density at 24 h was screened for accessing its morphological, biochemical and 16S r RNA sequencing analysis.

2.4. Extraction and Screening of herbal plants

About 10 herbal plants were selected for assessing their anti-UTI activity. Herbal plant materials were collected from the local market and were scientifically authenticated from SLS, SRTMU Nanded. Herbal plants like

Corriander sativum, *Abutilon indicum*, *Boerhavia diffusa*, *Plantago ovata*, *Bacopa monieri*, *Zingiber officinale*, *Azadiracta Indica, Ocimum sanctum*, *Cinnamomum cassia, Terminalia chebula* were selected under study. The extraction was done by using the Soxhlet apparatus for 5-6 cycles continuously with 10 gm extract in 200 mL solvents like ethanol, methanol and water, separately. After the extraction, the filtrates were then evaporated to yield pure extracts [20-22]. The percent yield was calculated using the formula-

(1) Percentage Yield (%) = Dry weight of extract/ Dry weight of plant material × 100

2.5. Anti-UTI activity

Each pure extract with solvents like ethanol, methanol and water of selected medicinal herbs were dissolved in DMSO and water to proceed for anti-UTI activity at a concentration of 1 mg/mL. Antimicrobial activity of all the pure extracts of selectively screened medicinal plants was performed by agar well diffusion method on Mueller Hinton Agar (MHA) plates. The test organism (UTI pathogen) was inoculated in nutrient broth and incubated overnight at 37◦C to adjust the turbidity to 0.5 McFarland standards giving final inoculum of 1.5×10^8 CFU/ml. A well of 6 mm was bored in the inoculated MHA plates with the help of sterile cork-borer (6 mm). Each well was filled with 100µl extracts from different plants. The standard antibiotics ampicillin, penicillin and chloramphenicol were used under study. Further, these plates were allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 h at 37◦C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the anti-UTI activity of tested herbal extracts, separately. The zone of inhibition (ZOI) was observed and measured in mm. Further, an attempt has been made to evaluate the combined effect of antibiotics with each herbal extract and the zone of inhibition was recorded.

3. Results

There are 20 UTI pathogens with different morphologies isolated from the UTI patients. Among these 20 uropathogens, the antibiotics resistant pathogens were screened for further study. Out of these 20 uropathogens 04 pathogens were revealed to be resistant for selective antibiotics under study. Further, these 04 uropathogens were assessed for its pathogenicity. Among these 04 uropathogens one of the organisms showed the highest growth at 24 h (Figure 1). The organism was carried forward for 16S r RNA sequencing techniques. Thus, obtained nucleotide sequence was deposited to NCBI to get the unique accession number. Based on the morphological, biochemical and 16S r RNA sequencing technique the organism was identified as *K. cowanii* (OQ 073698) with a unique accession number (Figure 2). The phylogenetic tree was constructed in Mega 11 software with the neighbor-joining method.

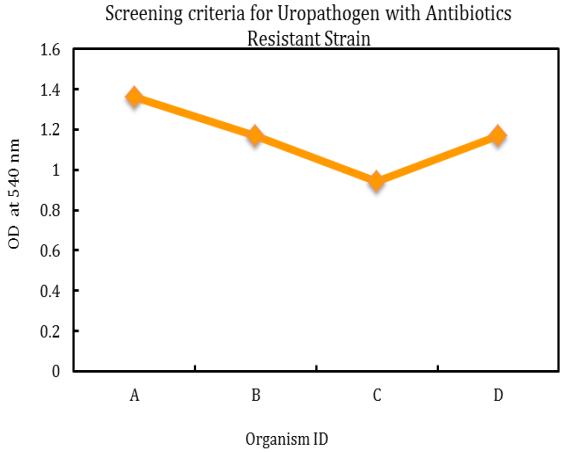

Fig.1. Screening criteria for Uropathogen

The bacterium *K. cowanii* (OQ 073698) is a Gram-negative *Bacillus* belonging to the order *Enterobacterales.* The bacterium *K. cowanii* (OQ 073698) was reported to be oxidase negative while motility, catalase, nitrate reduction, Simmons citrate, acid from glucose, gas from glucose, sorbitol, mannitol, lactose and sucrose test were reported to be positive (Table 1).

In the present study at the primary level, we have taken 10 different medicinal plants for soxhlet extraction in ethanol, methanol and water. From Table 2.it is clear that based upon the percent yield 03 herbal extracts were carried forward for anti-UTI activity against the isolated UTI pathogen *K. cowanii* (OQ 073698).

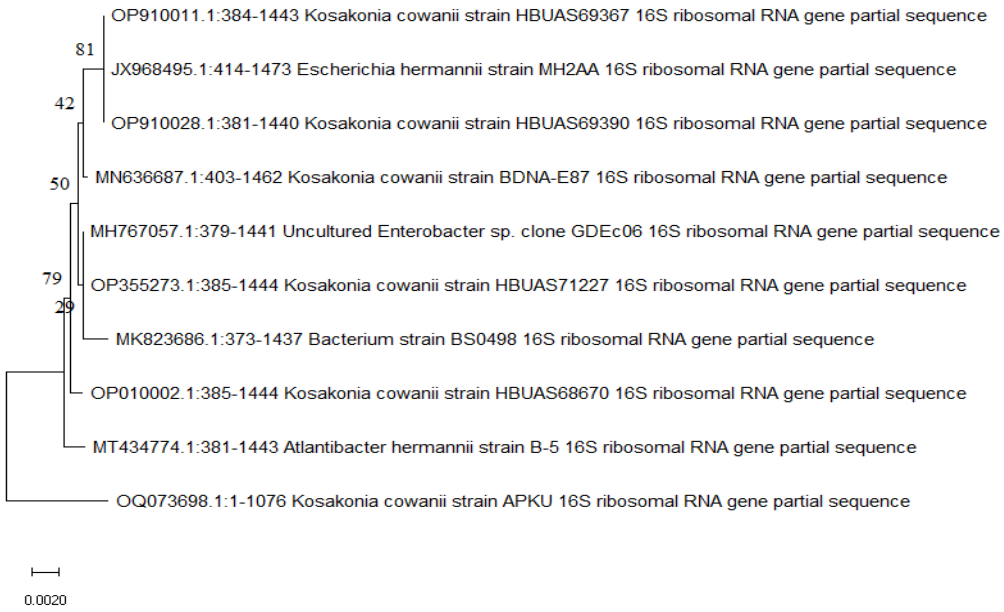

Fig.2. Phylogenetic tree of *K. cowanii* (OQ 073698) in Mega 11

Table 1. Biochemical characteristics of *K. cowanii* (OQ 073698)

Biochemical test	Result
Gram staining	Gram negative
Motility	+
Oxidase	-
Catalase	+
Nitrate reduction	+
Simons citrate (at room temperature)	+
Acid from glucose	+
Gas from glucose	+
Lactose	+
Sucrose	+
Mannitol	+
Sorbitol	+
Indole	-
Vogus Proskauer	-
Urease	-

Among all the extracts ethanolic extracts of *Boerhavia diffusa*, *Bacopa monnieri* and *Emblica officinalis* were effective in the anti-UTI activity. These extracts revealed the remarkable zone of inhibition against UTI pathogen *K. cowanii* (OQ 073698). The zone of inhibition against *K. cowanii* (OQ 073698) was reported with *Boerhaviadiffusa*1.6mm, *Bacopa monnieri*zone of inhibition were 1.8 mm and *E. officinalis* showed 1.8 mm while the standard antibiotics ampicillin 1.6, penicillin 1.7 mm and chloramphenicol 1.8 mm. The present study conclude the fact that *E. officinalis* showed the highest zone of inhibition against *K. cowanii* (OQ 073698) UTI pathogen when treated with individual ethanolic extracts (Figure 3a).

Table 2. The yield of different herbal extracts

Herbal extracts	Yield in		
	Ethanol /(10g)	Methanol /(10g)	water /(10g)
Coriander sativum	3.2	1.4	2.9
Abutilon indicum	2.8	3.4	1.6
Boerhavia diffusa	5.2	4.3	2.1
Plantago ovata	2.6	3.0	2.7
Bacopa monnieri	4.1	4.9	4.1
Zingiber officinale	3.2	2.2	3.6
Azadiracta indica	3.8	3.2	3.9
Ocimum sanctum	2.5	3.2	4.2
Cinnamomum cassia	3.1	3.9	2.1
Terminalia chebula	3.4	3.9	2.8
E. officinalis	5.1	4.7	2.8

However, one of the major findings from the study was reported that *E. officinalis* ethanolic extracts can replace the standard antibiotic chloramphenicol as the observed equal zone of inhibition with this complementary alternative medicine treatment we can minimize the adverse effect of antibiotics in certain cases. An attempt has been made in the present investigation to check the additive effect of antibiotics with the herbal extract. When standard antibiotic chloramphenicol (30 µg) was combined with the *E. officinalis* ethanolic extracts but no additive effects were observed as the zone of inhibition was reported 1.8 mm (Figure 3b).

Another result was reported from the present study; as the *K. cowanii* (OQ 073698) was ampicillin resistant earlier, though the individual ethanolic extracts of *E. officinalis* work but when it again combines with *Boerhavia diffusa* ethanolic extracts showed the zone of inhibition 2.0 mm. which was reported to be the highest zone of inhibition from the present investigation with all the herbal extracts tested under study (Figure 4). In present investigation triple effect of extracts with standard antibiotic ampicillin was effective to treat Urinary tract infection and which can minimize the adverse effect of antibiotics and certainly, we can minimize the emergence of antibiotics resistant pathogens.

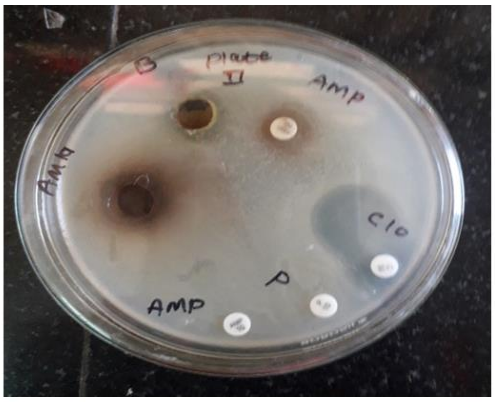

Fig. 4. Triple effect of herbal extract with std. antibiotics

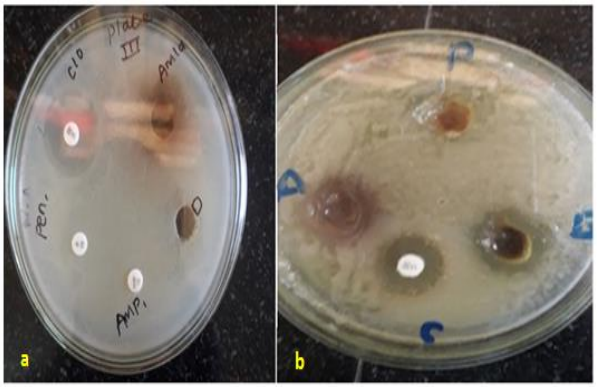

Fig. 3. Zone of inhibition against std. antibiotics and herbal extract (a), combine effect of antibiotics and herbal extract (b). (Amp-Ampicillin, Pen-Penicillin, Clo-Chloramphenicol, Amla- *E. officinalis*)

4. Discussion

This bacterium *K. cowanii* (OQ 073698) was dominant as a plant pathogen but it has only anecdotally been encountered as a human pathogen also. The *K. cowanii* bacterium was isolated from inside of the gallbladder and one bile sample during the surgery and identified based on MALDI TOF spectrometry [23, 24]. However, the bacterium *K. cowanii* was isolated from a patient with a urinary tract infection. To treat this UTI present study gives a flashlight on the effective herbal formulation and based on the severity of the infection it can combine with antibiotics to treat UTI effectively. Due to frequent exposure of antibiotics some cases bacteria develop resistance to the antibiotics. The emergence and exacerbation of the bacterial drug resistance problem, besides other problems of antibiotic therapy as the adverse effects of hepatoxicity, nephrotoxicity, ototoxicity, mutagenicity and carcinogenicity; immunosuppression; eradication of beneficial gut and mucosal surfaces flora and allergic reactions [25, 26], made most of the antibiotics worthless in treatment of many cases of UTIs and directed global attention towards finding new therapeutic alternatives. Therefore, the present study explores the best CAM approach with effective herbal extracts to treat urinary tract infections.

5. Conclusion

The present study concludes the fact that the UTI pathogen *K. cowanii* (OQ 073698) was effective when it was treated with ethanolic extracts of *E. officinalis* (1 mg/mL). Also, the same extracts can replace the chloramphenicol antibiotics as with the herbal we can minimize the side effects of the drug. Another major conclusion from the present investigation was when the Ampicillin antibiotic combined with the ethanolic extracts of *E. officinalis* and *Boerhavia diffusa* revealed the highest zone of inhibition 2.0mm. It indicates that the combined effect of both herbal extracts works better against the UTI pathogen.

Conflict of interest

The author declares no conflict of interest.

Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Author's contribution

A.P.K. Literature survey, designing the idea and experiments, analyzing the results, interpretation of results, and writing and reviewing the article. P.B.J. doing the experiments.

Human and animal studies

This research did not involve human participants and/or animals.

Informed Consent

The authors declare not used any patients in this research.

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References

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology 13 (5): 269. doi: https://doi.org/10.1038/nrmicro3432
2. Sheerin NS (2011) Urinary tract infection. Obstruction and Infection 39 (7): 384-389. doi: https://doi.org/10.1016/j.mpmed.2011.04.003
3. Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J, Gupta K, Trautner B (2018) Urinary tract infections, pyelonephritis, and prostatitis. Harrison's principles of internal medicine, 20th edn. McGraw-Hill Education, New York.
4. Hannan TJ, Totsika M, Mansfield KJ, Moore KH, Schembri MA, Hultgren SJ (2012) Host–pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. FEMS microbiology reviews 36 (3): 616-648. doi: https://doi.org/10.1111/j.1574-6976.2012.00339.x
5. Foxman B (2014) Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infectious Disease Clinics 28 (1): 1-13. doi: https://doi.org/10.1016/j.idc.2013.09.003
6. Olin SJ, Bartges JW (2015) Urinary tract infections: treatment/comparative therapeutics. Veterinary Clinics: Small Animal Practice 45 (4): 721-746. doi: https://doi.org/10.1016/j.cvsm.2015.02.005
7. Sheerin NS, Glover EK (2019) Urinary tract infection. Medicine 47 (9): 546-550. doi: https://doi.org/10.1016/j.mpmed.2019.06.008
8. Behzadi P (2020) Classical chaperone-usher (CU) adhesive fimbriome: uropathogenic *Escherichia coli* (UPEC) and urinary tract infections (UTIs). Folia microbiologica 65 (1): 45-65. doi: https://doi.org/10.1007/s12223-019-00719-x
9. Matulay JT, Mlynarczyk CM, Cooper KL (2016) Urinary tract infections in women: pathogenesis, diagnosis, and management. Current Bladder Dysfunction Reports 11: 53-60. doi: https://doi.org/10.1007/s11884-016-0351-x
10. Khameneh B, Iranshahy M, Vahdati-Mashhadian N, Sahebkar A, Bazzaz BSF (2019) Non-antibiotic adjunctive therapy: a promising approach to fight tuberculosis. Pharmacological research 146: 104289. doi: https://doi.org/10.1016/j.phrs.2019.104289
11. Bazzaz BSF, Fakori M, Khameneh B, Hosseinzadeh H (2019) Effects of omeprazole and caffeine alone and in combination with gentamicin and ciprofloxacin against antibiotic resistant *Staphylococcus aureus* and *Escherichia coli* strains. Journal of Pharmacopuncture 22 (1): 49. doi:

https://doi.org/10.3831%2FKPI.2019.22.006
12. Fazly Bazzaz BS, khameneh b, Zahedian MRO, Hosseinzadeh H (2018) In vitro evaluation of antibacterial activity of verbascoside, lemon verbena extract and caffeine in combination with gentamicin against drug-resistant *Staphylococcus aureus* and *Escherichia coli* clinical isolates. Avicenna Journal of Phytomedicine 8 (3): 246-253. doi: https://doi.org/10.22038/ajp.2018.15338.1607
13. Bazzaz BSF, Sarabandi S, Khameneh B, Hosseinzadeh H (2016) Effect of catechins, green tea extract and methylxanthines in combination with gentamicin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*:-combination therapy against resistant bacteria. Journal of pharmacopuncture 19 (4): 312. doi: https://doi.org/10.3831%2FKPI.2016.19.032
14. Baker JH, Qiu J, Grine K (2018) Role of complementary and alternative therapies in infectious disease. Primary Care: Clinics in Office Practice 45 (3): 533-539. doi: https://doi.org/10.1016/j.pop.2018.05.009
15. Mantzorou M, Giaginis C (2018) Cranberry consumption against urinary tract infections: clinical state-of-the-art and future perspectives. Current pharmaceutical biotechnology 19 (13): 1049-1063. doi: https://doi.org/10.2174/1389201020666181206104129
16. Poulios E, Vasios GK, Psara E, Giaginis C (2021) Medicinal plants consumption against urinary tract infections: a narrative review of the current evidence. Expert Review of Anti-infective Therapy 19 (4): 519-528. doi: https://doi.org/10.1080/14787210.2021.1828061
17. Loubet P, Ranfaing J, Dinh A, Dunyach-Remy C, Bernard L, Bruyère F, Lavigne J-P, Sotto A (2020) Alternative therapeutic options to antibiotics for the treatment of urinary tract infections. Frontiers in microbiology 11: 1509. doi: https://doi.org/10.3389/fmicb.2020.01509
18. Sabir S, Anjum AA, Ijaz T, Ali MA, Nawaz M (2014) Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. Pakistan journal of medical sciences 30 (2): 389. doi: https://pubmed.ncbi.nlm.nih.gov/24772149
19. Bauer A (1966) Antibiotic susceptibility testing by a standardized single diffusion method. Am J Clin Pathol 45: 493-496. doi:
20. Manandhar S, Luitel S, Dahal RK (2019) In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. Journal of tropical medicine 2019: 1-14. doi: https://doi.org/10.1155/2019/1895340
21. Keikhaie KR, Fazeli-Nasab B, Jahantigh HR, Hassanshahian M (2018) Antibacterial Activity of Ethyl Acetate and Methanol Extracts of Securigera securidaca, Withania sominefra, Rosmarinus officinalis and Aloe vera Plants against Important Human Pathogens. Journal of Medical Bacteriology 7 (1-2): 13-21. doi:
22. Fazeli-Nasab B, Rahnama M, Shahriari S (2019) The antimicrobial properties of hydro-alcoholic extracts of 29 medicinal plants on *E. Coli* and *Staphylococcus aureus* microbes. NFVM 1 (2): 1-15. doi: https://doi.org/10.35066/j040.2018.407
23. Berinson B, Bellon E, Christner M, Both A, Aepfelbacher M, Rohde H (2020) Identification of *Kosakonia cowanii* as a rare cause of acute cholecystitis: Case report and review of the literature. BMC Infectious Diseases 20: 1-4. doi: https://doi.org/10.1186/s12879-020-05084-6
24. Zandi S, Mariconti M, Zandi H, Jafari A, Hajimohammadi B, Eslami G, Vakili M, Sheykhzadegan M, Askari V, Hosseini SS (2022) Bacterial and Fungal Occurrence in Hydatid Cysts from Livestock in Central Iran. Veterinary Research Communications 2022: 1-10. doi: https://doi.org/10.1007/s11259-022-09959-8
25. Lopez A, Hudson J, Towers G (2001) Antiviral and antimicrobial activities of Colombian medicinal plants. Journal of ethnopharmacology 77 (2-3): 189-196. doi: https://doi.org/10.1016/S0378-8741(01)00292-6

26. Pájaro-González Y, Oliveros-Díaz AF, Cabrera-Barraza J, Cerra-Dominguez J, Díaz-Castillo F (2022) A review of medicinal plants used as antimicrobials in Colombia. Medicinal Plants as Anti-Infectives 2022: 3-57. doi: https://doi.org/10.1016/B978-0-323-90999-0.00005-7

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