GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Detection of the antibacterial role of *Achillea millefolium* against *Escherichia coli* isolated from urinary tract infections

Ahmed Azad Ahmed *

Van Yüzüncü Yıl University, Turkish public university located in Van.

GSC Biological and Pharmaceutical Sciences, 2024, 29(01), 026–032

Publication history: Received on 20 August 2024; revised on 28 September 2024; accepted on 30 September 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.1.0346

Abstract

Despite the fact that the pharmaceutical industry has developed numerous novel antibiotics over the last three decades, bacteria have developed resistance to these medications. Bacteria have the genetic potential to transfer and develop resistance to medications used as therapeutic agents. Therefore, the present study aimed to reveal the antibacterial role of *Achillea millefolium* against Escherichia coli isolated from urinary tract infections. Between February and July of 2024, 130 clinical samples were taken from individuals who were admitted to Kirkuk Hospital in Kirkuk City after visiting a specialist and being sent to the laboratory. Urine samples were taken from patients with UTI ranging in age from (1-60 years) of women, after which they were transferred directly to the laboratory to be cultured on the culture media, then *A. millefolium* extract was tested against Escherichia coli, compared with some standard antibiotics. Results showed that 57 (43.8%) of the total samples showed positive results for bacterial growth when cultured on the best culture media, which included blood and MacConkey agars. 73 (or 56.2%) of the total samples had results that showed no bacterial growth. E. coli showed total resistance (100%) to Ampicillin. While, a low sensitive toward Nalidixic acid (20.0%), Clindamycin (20.0%) 21.5%. Otherwise, E. coli showed a high sensitive to Gentamycin (87.7%), Imipenem (96.9%), and Amikacin (90.8%). While Tobromycin showed high sensitive 100.0% respectively. Based on the results, it was shown that the extract of A. millefolium at a dosage of 100 mg induced an inhibition diameter rate of 18.7±3.45, while at a concentration of 150 mg, it produced an inhibition diameter rate of 29.49±2.97. It is concluded from that the alcoholic extract of *A. millefolium* has an antibacterial effect and is very effective in treating cases of urinary tract infection resulting from infection with *Escherichia coli.*

Keywords: A. *millefolium*; *E. coli;* UTI; Antibiotic susceptibility.

1. Introduction

Even today, nearly every country's folk medicine relies heavily on herbal treatment [1]. Plants are major sources of medicine, and many medications used today are derived from them. Plant-based therapies are safe, cost-effective, and widely available [2]. Within the Asteraceae family, the genus Achillea comprises over 130 species of perennial herbs that are indigenous to the Northern Hemisphere, spanning from Europe to Asia. It thrives in dry or semi-arid environments at moderate temperatures [3]. The most notable aspect of this plant is its top flowers [4], which contain essential oil and have been utilized to treat influenza, bleeding, pain during menstruation, and vomiting [5]. A. millefolium tea can also be used to treat gastrointestinal issues such as indigestion, gas, nausea, vomiting, and stomachache. In an earlier clinical study, volunteers were assigned at random, and neither the researchers nor the participants knew who received the drug. This study discovered that A. millefolium flower powder alleviated pain in primary dysmenorrhea. Additionally, millefolium tea alleviates chest pain, bloating, and diarrhea. A clinical study discovered that tea prepared with A. millefolium flower powder effectively reduces the severity of dysmenorrhea pain [6]. Numerous substances are present in it, such as beta-pinene, borneol, camphor, eucalyptol, alpha terpineol, artemetin, apigenin, volatile oil, salicylic acid, azulene, chamazulene, sesquiterpenoids, and so on [7-8]. Several

* Corresponding author: Ahmed Azad Ahmed.

phytochemical constituents have been identified in the essential oil extracted from Achillea millefolium L. These include borneol, 1.8-cineole, eucalyptol, limonene, sabine, terpin4-ol, terpineol, achillin, millefin and millefolide (sesquiterpene lactones), azulene and chamazulene, and isoartemisia ketone. These phytochemical constituents have been investigated further after ongoing phytochemical studies of conventional and folklore remedies [9–10]. In example, it was proposed that the presence of different secondary metabolites including phenols and flavonoids contributes for yarrow's antibacterial properties [11]. Different extracts of A. millefolium aerial parts were reported to be efficacious against gram positive and negative bacteria, Aspergillus spp., and Candida spp. [12]. Methanol extract is effective against H. pylori with a MIC of 50 µg/mL [13]. Therefore, the present study aimed to reveal the role of A. millefolium as an antibacterial against E. coli isolated from UTI.

2. Material and methods

2.1. Specimen Collection

From February to July 2024, 130 clinical samples were obtained from Kirkuk Hospital in Kirkuk city from individuals who had been admitted and hospitalized after meeting with a specialist doctor and were referred to the laboratory. Urine samples were taken from patients with UTI ranging in age from (1-60 years) of women, after which they were transferred directly to the laboratory to be implanted on the culture media.

2.2. Bacterial Identification

Bacteria were diagnosed based on the following aspects:

2.2.1. Morphological diagnosis and media characteristics

Based on the culturing features of the *E. coli* colonies developing on blood agar and mannitol salt agar media, the colonies were diagnosed and incubated for 24 hours at 37 ^{0}C.

2.2.2. Direct examination

By using a microscope to examine the morphological characteristics of germ cells—specifically, how they contacted the gram stain, which indicates the kind of interaction as well as the shape and arrangement of the germ cells—bacterial colonies were found.

2.2.3. Biochemical reaction and motility test

Numerous biochemical tests, such as the H2S production, methyl red, citrate, urease, Voges-proskauer, catalase, oxidase, and indole test, were carried out in order to identify and diagnose bacteria.

2.2.4. Identification of bacteria isolates via VITEK2

VITEK 2 is the next version of the gold standard for microbiological detection, incorporating improved colorimetric technology. The following processes were completed in accordance with the directions provided by the manufacturer (Biomerieux).

2.3. Extract preparation

10 grams of dried yarrow flower powder were suspended in 150 milliliters of distilled 100% ethanol and whirled on a magnetic stirrer with no heating until completely blended. The mixture was then frozen at 4 degrees Celsius for 24 hours, and both infusions were filtered via multiple layers of gauze and Whatman No. 1 filter paper. After drying, the two dried extract samples were weighed and then kept at 4 degrees Celsius in dark-colored bottles lined with aluminum foil to protect against the detrimental effects of light. Following the preparation of the sample stock solution, which contained 100 and 150mg/ml of alcoholic extract (one gram of dried extract suspended in five milliliters of dimethyl sulfoxide), the alcoholic stock solution was pasteurized at 62 degrees Celsius for ten minutes [14]. It was then evaluated against Escherichia coli and compared with a few common antibiotics [15].

3. Results and discussion

3.1. Samples distribution

The current study included 130 urine samples collected from patients with urinary tract infection (table 1). The data revealed that 57 (43.8%) of total samples produced positive outcomes for bacterial growth when cultured on appropriate cultured media including blood and MacConkey agars. 73 (56.2%) of the total samples yielded results that were negative for bacterial growth.

Table 1 Distributed of study samples according to UTI

	No. (%) +ve culture	**No. (%) -ve culture**	**Total No.(%)**
Patients	57(43.8%)	73(56.2%)	130 (100.0%)

The growth culture rate found in women with UTI is roughly comparable to that found in earlier research conducted in Iraq by [16] and [17], which found growth culture at 45.8% and 55.4%, respectively. The data presented by [18], who recorded growth culture with only 22.7%, is in conflict with the results. Differences in sample size, regional variance, awareness, and predisposing factors could all be contributing causes to the inconsistent results [19]. Negative growth can be caused by a variety of factors, including the overuse of antibiotics or other infectious agents including fungus, viruses, or anaerobic bacteria. Because they require specific media and isolation conditions, these anaerobic bacteria are challenging to isolate using the same techniques as aerobic bacteria [20].

3.2. Identification

On MacConkey agar, Escherichia coli was identified by the formation of small, dry pink colonies. On EMB, the bacteria was identified by its green color (fig. 1), and when grown on the EMB media, it appeared to have a green metallic sheen. The IMVC test revealed that the bacteria was negative for Lactose Fermentation, Urease Test, Oxidase, and Voges–Proskauer Test, but positive for Indole, Citrate Test, Methyl Red, and motility. One of the most persistent illnesses in males of all ages is UTIs [21]. The recent data also showed that the rate of E. Coli infection was 55.6%. It has been well-documented that E. coli is the primary cause of UTIs globally. A study conducted in a separate nation revealed an E. coli isolation rate of 45.67% [18].

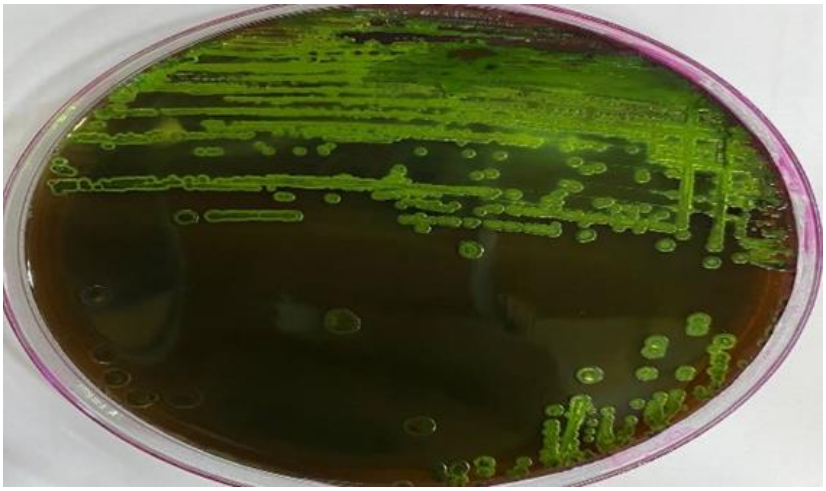

Figure 1 *E. coli* colonies on EMB agar.

3.3. Antibiotic susceptibility test

E. coli showed total resistance (100%) to Ampicillin. While, a low sensitive toward Nalidixic acid (20.0%), Clindamycin (20.0%) 21.5%. Otherwise, E. coli showed a high sensitive to Gentamycin (87.7%), Imipenem (96.9%), and Amikacin (90.8%). While Tobromycin showed high sensitive 100.0% respectively, as shown in table (2).

Table 2 Antibiotic susceptibility test of *E. coli*

Antibiotics	Sensitive %	Intermediate %	Resistant %
AMP	0.0	0.0	100.0
VN	21.5	3.1	75.6
DA	20.0	0.0	80.0
TMP	26.2	3.1	70.3
CAZ	30.8	6.2	63.0
CTX	13.8	7.7	78.5
CFM	16.9	6.2	77.9
CN	87.7	3.1	9.2
IMI	96.9	0.0	3.1
NA	20.0	1.5	78.5
CIP	21.5	9.2	69.3
LEV	24.6	10.8	64.6
AZT	49.2	15.4	35.4
AK	90.8	0.0	9.2
TOB	100	0.0	0.0

AMP= Ampicillin, VN= Vancomycin, DA=, Clindamycin, TMP=Trimethoprim, CAZ= Ceftazidime, CTX=, Cefotaxime, CPM=Cefepime, CN=Gentamicin, IMI=Imipenem, NA=Nalidixic acid, CIP=Ciprofloxacin, LEV= Levofloxacin, AZT = Azithromycin, AK=Amikacin, TOB = Tobramycin.

According to the current study's findings, all of the E. Coli isolates were very susceptible to Gentamycin, Imipenem, Amikacin, and Tobramycin, but showed 100% resistance to Ampicillin. The study's findings were not consistent with [23], where the resistance ratios for tetracycline and ampicillin were 50%, but the results were nearly identical in terms of their sensitivity to gentamycin, trimethoprim, and chloramphenicol, which were 100%, 75%, and 50%, respectively. In the study by [24], the bacteria were 100% responsive to the antibiotic amoxicillin, and the findings were also different. Comparable results were obtained for gentamycin, with a sensitivity of 100% and 71.43%, respectively. The presence of genes encoding particular enzymes, such as β-lactamases, which break down resistant antibiotics, the acquisition of pumps by bacteria that aid in destroying the antimicrobial element in the cell before the antibody reaches the target site as well as currency, the acquisition of multiple genes from the metabolic pathway by bacteria that alters the bacterial cell wall, or the acquisition of a genetic mutation by bacteria that prevents the antibacterial agent from reaching the target site within the cell are some of the factors that can lead to antibiotic-resistant bacterial isolates [25]. Additionally, the results of this study corroborate those of Abdul-Lateef [26], who said that 90% of patients did not have ampicillin resistance. Because βlactamases are produced, gram-negative bacteria can exhibit resistance to β-lactam drugs. (ESBLs) are a group of enzymes that are capable of hydrolyzing several β-lactam antibiotics, such as cefoxitin but not penicillin or cephalosporins like ceftazidime, cefotaxime, and ceftriaxone [27].

3.4. The inhibitory effect of ethanol extract *A. millefolium*

The results show ability of *A. millefolium* for bacteria inhibition according to concentration. Table (3) shows that the rate of inhibition diameter caused by the alcoholic extract of A. millefolium at a concentration of 100 mg was 18.7±3.45, while the rate of inhibition diameter caused by the alcoholic extract of A. millefolium at a concentration of 150 mg was 29.49±2.97.

Table 3 the effect of ethanoic extract of *A. millefolium* at concentration 100 and 150mg

Extract **Bacteria isolates**	***A. millefolium***	
	100mg	150mg
S1	17.4	25.6
S2	15.3	28.8
S3	19.7	27.1
S4	15.8	26.9
S5	21.4	31.4
S6	14.2	35.1
S7	21.8	30.5
S8	24.8	27.3
S9	20.5	29.5
S10	16.1	32.7
Mean±SD	18.7±3.45	29.49±2.97

Similar findings to those shown here have been reported in a number of investigations into the antibacterial qualities of this species [28–29]. One distinction is that two investigations using ethanol extract of yarrow discovered inhibitory zones against E. coli in disc diffusion assays; in contrast, two other research using aqueous extracts of the plant's flowers, leaves, roots, and shoots as well as an independent investigation using essential oil and methanolic extracts did not find the same results. These inconsistent findings could be the result of geographical variations in the chemical composition of the plants or various extraction techniques. It is commonly recognized that Yarrow is a polyploid complex that is varied and likely made up of several dozen species with different biochemical makeups. There is a good amount of known information regarding this complex's biochemical diversity, and the antibacterial action that has been reported may be due to phenolic substances such flavonoids and phenolcarbonic acids [30]. Since A. millefolium oil has demonstrated efficacy against a wide range of microorganisms, including Candida spp., S. pneumoniae, C. perfringens, Acinetobacter l., and Escherichia coli, its application in antibiotics has the potential to completely transform the pharmaceutical sector [31,32].

4. Conclusion

It is concluded that the most effective antibiotic on *Escherichia coli* is Tobromycin. On the other hand, the study revealed that the alcoholic extract of *A. millefolium* has an antibacterial effect and is very effective in treating cases of urinary tract infection resulting from infection with *Escherichia coli*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Ahmed QA, Rahim SM, Hameed AK. The effect of hydroxytyrosol (hxt) and a local olive oil extract on the level of hepcidin hormone and pathological histological changes with iron deposition in the aorta resulting from induced hyperlipidemia in male rats. Plant Archives. 2020;20(2):1895-902.

[2] Ahmed QA, Abdullah SI, Taher HM. Effects of the alcoholic extract of ginseng roots and carob fruits in comparison with vitamin E in improving the efficiency of the male reproductive system of albino rabbits. Caspian Journal of Environmental Sciences. 2023 Oct 1;21(4):815-26.

[3] Ali S. I., Gopalakrishnan B. and Venkatesalu V. Pharmacognosy, Phytochemistry and Pharmacological Properties of Achillea millefolium L.: A Review. Phytother. Res. 2017; 9: 1-23.

[4] Fierascu I, Ungureanu C, Avramescu SM, Fierascu RC, Ortan A, Soare LC, et al. In vitro antioxidant and antifungal properties of Achillea millefolium L. Rom Biotechnol Lett. 2015;20(4):10626-36.

[5] Benedek B, Rothwangl-Wiltschnigg K, Rozema E, Gjoncaj N, Reznicek G, Jurenitsch J, et al. Yarrow (Achillea millefolium L. s.l.): pharmaceutical quality of commercial samples. Pharmazie. 2008;63(1):23-6.

[6] Jenabi E, Fereidoony B. Effect of Achillea millefolium on relief of primary dysmenorrhea: a double-blind randomized clinical trial. J Pediatr Adolesc Gynecol. 2015;28(5):402-4

[7] Falconieri D, Piras A, Porcedda S, Marongiu B, Gonçalves MJ, Cabral C, et al. Chemical composition and biological activity of the volatile extracts of Achillea millefolium. Nat Prod Commun. 2011;6(10):1527-30.

[8] Benedek B, Kopp B. Achillea millefolium L. s.l. revisited: recent findings confirm the traditional use. Wien Med Wochenschr. 2007;157(13-14):312-4.

[9] Jamal Q, K. & AH., G. (2006). Studies on hepatoprotective, antispasmodic and calcium antagonist activities of the aqueous-methanol extract of Achillea millefolium. Phytother Res 2006, 20(7), 546-551.

[10] Kaur, M., Singhal, V. K., & Singh, J. (2017). Use of some ethnomedicinal herbs by the natives of Solang valley, Kullu district, Himachal Pradesh. Int J Pharm Pharm Sci, 9(9), 222-227.

[11] Faiku, F.; Haziri, A.; Mehmeti, I.; Mehmeti, A.; Hoti, G. In vitro antibacterial activity of different solvent extracts of Achillea millefolium (L.) growing wild in Kosovo. Fresenius Environ. Bull. 2018, 27, 3878–3883.

[12] Stojanovic, G.; Radulovic, N.; Hashimoto, T.; Palic, R. In vitro antimicrobial activity of extracts of four Achillea species: The composition of Achillea clavennae L. (Asteraceae) extract. J. Ethnopharmacol. 2005, 101, 185–190.

[13] Mahady, G.B.; Pendland, S.L.; Stoia, A.; HamilL F.A.; Fabricant, D.; Dietz, B.M.; Chadwick, L.R. In vitro susceptibility of Helicobacter pylori to botanical extracts used traditionally for the treatment of gastrointestinal disorders. Phytother. Res. 2005, 19, 988–991.

[14] Al – Neman, A.Y.S. (1998). The molecular effect of some plant extracts on the Growth and metabolism of some Gram positive and Gram negative Bacteria .Ph. D. thesis, College of Science, University of Mosul, Mosul, Iraq. (in Arabic)

[15] Collee, J. G.; Fraser, A. G.; Marmion, B. P.; Simmons, A. (1996). "Mackie and McCartney Practical Medical Microbiology". 14th ed., Churchill Livingstone, New York.

[16] Khanam R. A., Sharif A., Sharin L., Sarker S., Jahan H., Yusuf M.A. Clinical Utility of Antibiotic against Uropathogen Isolated at a Tertiary Care Hospital of Dhaka City". American Journal of Microbiological Research, 2018; 6(2): 63-66.

[17] Al-Asady F. M., Al-Saray D. A., & Al-Araji A. E. "Screening of urinary tract bacterial infections and their antibiogram among non-pregnant women admitted to Al-Sadiq hospital, Iraq ". In AIP Conference Proceedings , 2022; 2386 (1): 1-6.

[18] M.Kibret, B. Abera, "Prevalence and antibiogram of bacterial isolates from urinary tract infections at Dessie Health Research Laboratory, Ethiopia". Asian Pacific journal of tropical biomedicine, 2014; 4(2): 164- 168.

[19] Edae M., Teklemariam Z., Weldegebreal F., & Abate D. Asymptomatic bacteriuria among pregnant women attending antenatal care at Hiwot Fana specialized university hospital, Harar, eastern Ethiopia: magnitude, associated factors, and antimicrobial susceptibility pattern". International Journal of Microbiology, 2020; 2020: 8.

[20] Fareid M.A. Frequency and susceptibility profile of bacteria Causing urinary tract infections among women". New York Science Journal, 2012; 5(2): 72- 81.

[21] Kleina R. D., and Scott J. H. Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies. Nat Rev Microbiol. 2020; 18(4): 211–226.

[22] Armanullah M, Anjay, Kumar P, Kumari S, Kaushik P, Archana S, Das Arya K. Prevalence of multi-drug resistant (MDR) Escherichia coli in bovine clinical samples. Int J Curr Microbiol App Sci. 2018;7:1476-1485.

[23] Messele, Y. E., Abdi, R. D., Yalew, S. T., Tegegne, D. T., Emeru, B. A., & Werid, G. M. (2017). Molecular determination of antimicrobial resistance in Escherichia coli isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. Annals of clinical microbiology and antimicrobials, 16(1), 55.

[24] Rahman, M. A., Rahman, A. K. M. A., Islam, M. A., & Alam, M. M. (2017). Antimicrobial Resistance Of Escherichia coli Isolated From Milk, Beef And Chicken Meat In Bangladesh. Bangladesh Journal of Veterinary Medicine, 15(2), 141-146.

[25] Kapil, A. (2005). The challenge of antibiotic resistance: need to contemplate. Indian Journal Medical Research, 121(2), 83-91.

[26] Abdul-Lateef, L. A. (2011). Phylogeny and pathogenicity Island among Escherichia coli islated from clinical cases. thesis. Ph.D. college of Medicine. University of Babylon.

[27] Al-Hadithi H. A. A. (2018). Molecular detection of Hemolycin in Escherichia coli and attempt to inhibition by using the Probiotics. Tikrit J. Pur Sci., 23(6) 79-90.

[28] Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HS: Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achilleamillefoliumsubsp. MillefoliumAfan. (Asteraceae). Journal of Ethnopharmacology 2003, 87:215-220.

[29] Benedek B, Gjoncaj N, Saukel J, Kopp B: Distribution of the phenolic compounds in Middleeuropean taxa of the AchilleamillefoliumL. aggregate. Chemistry and Biodiversity 2007, 4:849-857.

[30] Tajik H, Jalali FSS, Sobhani A, Shahbazi Y, Zadeh MS: In vitro assessment of antimicrobial efficacy of alcoholic extract of Achilleamillefoliumin comparison with penicillin derivatives. Journal of Animal and Veterinary Advances 2008, 7:508-511.

[31] Candan F et al (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). Journal of Ethnopharmacology. 87,pp 215-220

[32] Ahmed QA. Studying the possibility of using olive oil and its active components together with atorvastatin in the treatment of biochemical parameters and iron deposition in heart tissue with lung histopathology conditions induced by hypercholesterolemia in male rod. GSC Biological and Pharmaceutical Sciences. 2023;24(2):134-47.