In vitro Assessment of the Antimicrobial Potential of Some Commercial Herbal Products

Zahraa A. Hashim
Clinical Laboratory Sciences Department, College of Pharmacy, Mosul University, Mosul, Iraq.
Corresponding author: hashimz@uomosul.edu.iq

This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/)

Received 20-10-2022 Accepted 21-11-2022

ABSTRACT:

Natural plant products have been widely used as an alternative approach to treat microbial infections to overcome issues of antibiotics-associated side effects and the emergence of resistant pathogens. One of the most popular infections causing significant morbidity and death toll is urinary tract infection. This study is designed to evaluate the antimicrobial and antibiofilm potential of four commercial herbal products against five uropathogenic clinical isolates. *In vitro*, well diffusion assay and crystal violet staining techniques were used to evaluate the antimicrobial and antibiofilm effects respectively. Antimicrobial effect was reported for Renalka™ against *Enterococcus faecium*, ROWAtinex® against *Staphylococcus aureus* and *E. faecium* and UROCLEAR against *Candida albicans*. A significant antibiofilm effect, semi-quantified by stained biomass, was reported for all of the products included in the study against the tested pathogens but to a varying degree. Medicinal herbal products can exert a substantial prophylactic antibiofilm effect in addition to their beneficial health effects in treating urological pathologies.

Keywords: Antibiofilm; Crystal violet; Herbal products; Well-diffusion assay; Uropathogens.

تقييم في المختبر لإمكانية مضادات الميكروبات لبعض المنتجات العشبية التجارية

الخلاصة

تم استخدام المنتجات النباتية الطبيعية على نطاق واسع كنهج بديل لعلاج الالتهابات الميكروبية للتغلب على مشاكل الآثار الجانبية المرتبطة بالمضادات الحيوية وظهور مسببات الأمراض المقاومة. تعد عدوى المسالك البولية واحدة من أكثر أنواع العدوى شيوعًا التي تسبب المراضة والوفيات الكبيرة. صممت هذه الدراسة لتقييم إمكانات مضادات الميكروبات ومضادات الغشاء الحيوي لأربعة منتجات عشبية تجارية مقابل خمس عزلات إكلينيكية مسببة للأمراض البولية. تم استخدام تقنيات الانتشار في المختبر وتقنيات تلطيخ البنفسج الكريستالي لتقييم تأثير مضادات الميكروبات والأغشية الحيوية على التوالي. تم الإبلاغ عن تأثير مضاد للميكروبات لـ Renalka™ ضد *Enterococcus faecium* و ROWAtinex® ضد *Staphylococcus aureus* و *E. faecium* و UROCLEAR ضد المبيضات البيضاء. تم الإبلاغ عن تأثير مضاد حيوي كبير ، شبه مُحدد بواسطة الكتلة الحيوية الملطخة ، لجميع المنتجات المدرجة في الدراسة ضد مسببات الأمراض المختبرة ولكن بدرجات متفاوتة. يمكن للمنتجات العشبية الطبية أن تمارس تأثيرًا وقائيًا كبيرًا للمضادات الحيوية بالإضافة إلى آثارها الصحية المفيدة في علاج أمراض المسالك البولية.

الكلمات المفتاحية: مضاد الغشاء الحيوي، الكريستال البنفسجي، منتجات عشبية، فحص انتشار، ممرض المسالك البولية.

INTRODUCTION:

Using herbal products in pharmacy and medicine has been expanded extensively over the last few decades, though their therapeutic virtues were practically concerned by our ancestors centuries ago. By definition, herbal medicines are those parts of plants that are intended for use for their aroma, flavour, and most relatively for their therapeutic characteristics (1). A report

from the organization of world health (WHO) has depicted the effective contribution of medicinal plants to primary health care (2). These herbal products have been shown effective in alleviating different health disorders ranging from simple flu-like symptoms to sever, life-threatening illnesses such as malignancies (1). Among these major ailments are urinary tract disorders such as renal spasm, urolithiasis, renal stones, and urinary tract infection (UTI). UTI is one of the most common infections with gender and age variation (3). Almost 50% of females reported one incidence of UTI with or without recurrent occurrence with a rate globally estimated to be of minimum 250 million (4). Bacterial adherence to the uroepithelial cells and subsequent biofilm formation is considered a key virulence of this infection (5). Many different microbes can cause UTIs with around 75% attributed to *Escherichia coli* while *Pseudomonas* and *Enterococcus* spp. are predominant in complicated UTIs. Strains of *Staphylococcus aureus* and *Candida albicans* are also isolated from patients with UTIs (6). Because of antibiotics-associated side effects and the emergence of problematic multi-drug resistant strains, a shift towards alternative remedies has brought the old plant-based medication into the contemporary scene. Many pharmaceutical polyherbal preparations have been formulated for urinary tract disorders (7,8). Their therapeutic efficacy has been attributed to the phytochemical constituents of these herbal products (9).

In this context and since microbial adherence is the pioneering step in the uropathogenesis of UTIs, a hypothesis that herbal-based formulation might adopt a role in the interference with microbial biofilm development *in vitro* has been made. Thereby, the antimicrobial and antibiofilm potential of some commercial herbal products, formerly intended for renal disorders, against uropathogenic isolates were studied *in vitro*.

MATERIALS AND METHODS

The herbal products used in this study were purchased from the local community pharmacies in Mosul/Iraq. The selection was made depending on their therapeutic indications for urinary tract disorders as per the product's label recommendation. No product had surpassed the expiry date on the package. All of the products were intended for the oral port of use. Products were stored at 4 ˚C until use. Products were randomly numbered from one to four as follows: 1: Renalka™, 2: ROWAtinex®, 3: Cystone®, 4: UROCLEAR.

Microbial isolates

The five microbial species used in this study were obtained from culture stocks from the Microbiology Laboratory of the College of Pharmacy/Mosul University. These clinical isolates were previously isolated from urine samples of patients complaining of urinary tract infections. The isolates were two Gram negative; *P. aeruginosa* and *E. coli*, two Gram positive; *E. faecium* and *S. aureus* and one yeast; *C. albicans*. The pure isolates were cultured on the appropriate agar medium and kept at 4 ˚C until use (a maximum of 2 weeks).

Antimicrobial potential of the herbal products

The antimicrobial activity of the four commercial herbal products was assessed by employing *in vitro* well diffusion assay (10). Briefly, nutrient agar (Sigma-Aldrich) and Sabouraud's dextrose agar (Sigma-Aldrich) plates were prepared. Bacterial overnight nutrient broth cultures of *S. aureus*, *E coli*, *P. aeruginosa*, and *E. faecium* were centrifuged and the bacterial pellets were obtained. The individual pellet of each bacterial isolate was then resuspended in phosphate buffer saline (PBS) and the concentration was adjusted to a final absorbance (600 nm) of 0.08-0.1 equivalent to 1 x 10^8 colony forming unit (CFU)/mL. Overnight culture of *C. albicans* in Sabouraud's broth (Sigma-

Aldrich) was treated similarly and the absorbance (600 nm) was adjusted to 0.1 corresponding to around 1 x 10^7 CFU/mL. Triplicate of nutrient and Sabouraud's plates were lawn cultured with the corresponding microbial suspension. The concentrations used to assess the antimicrobial effect were the daily dose recommended by the products' manufacturers. Regarding solid products, the daily doses were measured and dissolved in PBS. Five wells were made on the surface of the lawned plates and 200 µL of the herbal products were pipetted into the corresponding wells. Gentamicin (10 mg/mL) and vancomycin (8 mg/mL) were used as the positive control for the Gram-negative and the Gram-positive bacteria respectively. Fluconazole (10 mg/mL) was employed as the positive control for *C. albicans*. Plates were then incubated at 37 ˚C for 24 h under aerobic conditions. After the 24 h incubation, plates were monitored for the no-growth zone around the induced wells.

Antibiofilm activity of the herbal products

To assess the latent antibiofilm activity of the selected herbal products, the crystal violet staining assay was adopted (11). Briefly, a standardized microbial culture of the test microorganisms was prepared equivalent to a final concentration of 1 x 10^8 CFU/mL as explained above. An inoculum volume of 100 µL was pipetted into the respective wells of sterile polystyrene flat bottomed 96-well plate in triplicates and followed by the addition of 100 µL of the prepared herbal products. For each test, appropriate growth control was included. The plate was then incubated under an aerobic atmosphere at 37 ˚C for 24 h. At the end of the incubation period, wells' contents were gently pipetted out followed by washing the wells with distilled water three times to eradicate slackly or unattached cells. The wells were then air-dried and fixed with 96% methanol for 10 min. Following emptying the wells, the adherent cells were stained by pipetting a volume of 200 µL of 0.1 % crystal violet solution for 15 min. Stained wells were then emptied and rinsed with tap water to decant surplus stain. The inoculated wells were then air-dried and the stained biomass cells were resolubilized by pipetting 200 µL of 30% acetic acid into the individual well. Using a spectrophotometer (Cole-Parmer), the absorbance of the plate was read at 600 nm. The percentage of biofilm formation inhibition was calculated conferring to the following equation (12):

$$\% \text{ inhibition} = [(\text{absorbance}_{\text{growth control}} - \text{absorbance}_{\text{sample}}) / \text{absorbance}_{\text{growth control}}] \times 100$$

; where absorbance is the mean reading of three repeats.

RESULTS

Four products were purchased and labelled numerically from 1 to 4; 1: Renalka™, 2: ROWAtinex®, 3: Cystone®, 4: UROCLEAR. Their herbal constituents are listed in table 1. Of the four products included, one was in liquid form as a syrup (Renalka™), one as gelatine capsule (ROWAtinex®) while the two others were dry power in tablet and sachet from (Cystone® and UROCLEAR, respectively).

Table 1. Description of the active constituents of each herbal product included in the study

Product name and label number	Dosage form	Composition	Concentration /dose	Indications as per the product's

				leaflet
1. Himalaya Renalka™	Syrup	Cretaeva magna Hemidesmus indicus Cyperus rotundus Vetiveria zizanioides Asparagus racemosus Elettaria cardamomum Kshara parpati Tribulus terrestres Trikatu	50 mg 50 mg 50 mg 50 mg 50 mg 16.5 mg 75 mg 50 mg 16.5 mg	Assist in dissolution and expulsion of stones in the renal system Treatment of urinary tract spasm and inflammation associated with urolithiasis.
2. ROWAtinex®	Capsule	α-Pinene β-Pinene Camphene Borneol Anethol Cineol Fenchone Olive Oil ad	24.8 mg 6.2 mg 15 mg 10 mg 4 mg 3 mg 4 mg 100 mg	Renal disorders, urolithiasis and urinary tract infections
3. Cystone®	Tablet	Extracts Didymocarpus pedicellate Rubia cordifolia Cyperus scariosus Achyranthes aspera Onosma bracteatum Vernonia cinerea	65 mg 16 mg 16 mg 16 mg 16 mg 16 mg 49 mg	Urolithiasis: phosphate stone, oxalate stone, uric acid and urate stone, prevention of post-operative recurrence of calculi, crystalluria, mixed crystals-glycolic acids. Adjuvant in: urinary tract

		Saxifraga ligulata Powder Hajrul yahood bhasma Shilajeet (purified)	16 mg 13 mg	infections, burning micturition, urinary tract infections during pregnancy, cystitis. Sialolithiasis. Urinary incontinence in women.
4. UROCLEAR	Sachet (powder)	Cranberry extract Potassium citrate D-mannose	90 ng 30 mg 1000 mg	Support kidney function, prevent urinary tract infection,

Well diffusion assay

The *in vitro* antimicrobial potential of the herbal products was measured in this study with the agar well diffusion assay. Figure 1 and Table 2 illustrate the reported antimicrobial effect. Inhibition of the staphylococcal growth was monitored in response to the inoculation of ROWAtinex® with a zone of inhibition comparable to that caused by the positive control (30 ± 3.0 and 32.4 ± 2.2 mm, respectively). The same product was shown able to effectively inhibit the growth of *E. faecium* with an inhibition zone of 25.3 ± 1.1 mm. The latter pathogen responded sensitively to the growth inhibitory effect of Renalka™ showing a zone of inhibition of 35 ± 1.0 mm. Though not significant, UROCLEAR demonstrated the ability to hinder the growing of the infective yeast *C. albicans* (14.5 ± 2.0 mm). Cystone® did not succeed in suppressing the growth of any of the test microbes. The Gram negative rods were found resistant to the four tested herbal products.

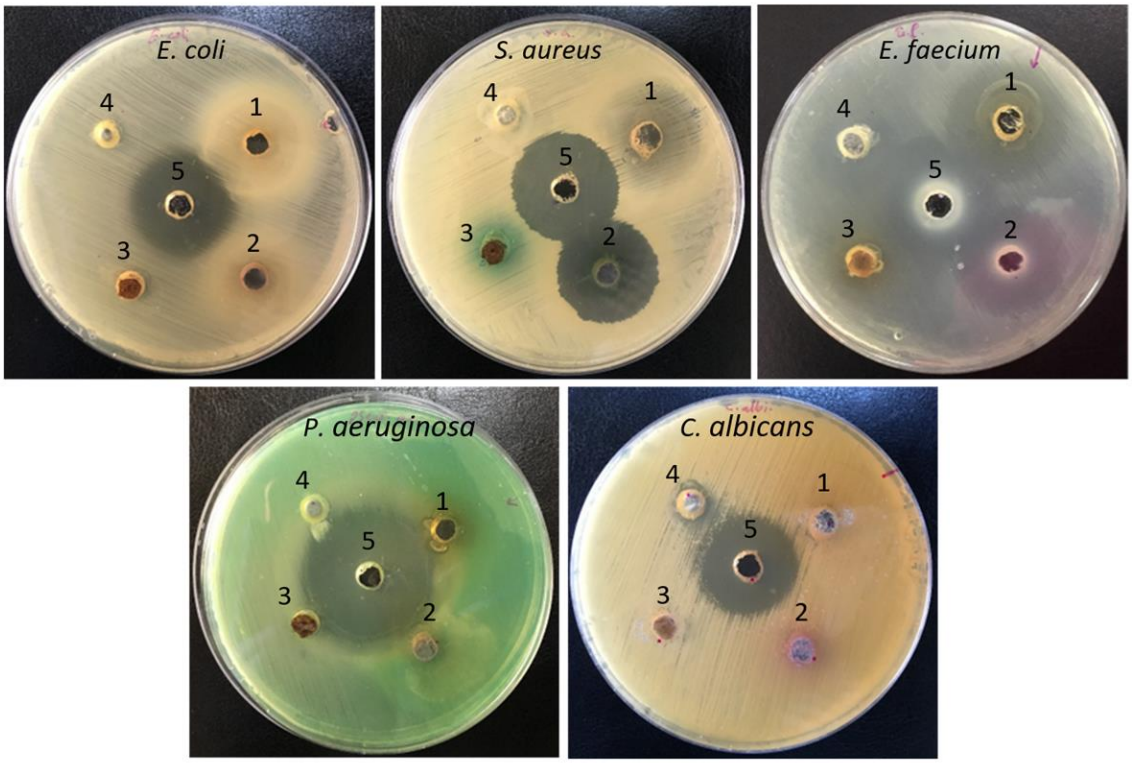

Figure 1. *In vitro* antimicrobial activity of the herbal products assessed by using well diffusion assay. 1: Renalka™ , 2: ROWAtinex®, 3: Cystone®, 4: UROCLEAR, 5: positive control.

Table 2. Zone of inhibition diameters (mm) of the daily dose concentration of the herbal products tested (expressed as mean ± standard deviation)

Test Microorganism	**Herbal Product**				
	Renalka™	ROWAtinex®	Cystone®	UROCLEAR	Positive control
C. albicans	0 ± 0	0 ± 0	0 ± 0	14.5 ± 2.0	25 ± 1.5
S. aureus	0 ± 0	30 ± 3.0	0 ± 0	0 ± 0	32.4 ± 2.2
E. coli	0 ± 0	0 ± 0	0 ± 0	0 ± 0	24.1 ± 2.0
P. aeruginosa	0 ± 0	0 ± 0	0 ± 0	0 ± 0	42 ± 3.5
E. faecium	35 ± 1.0	25.3 ± 1.1	0 ± 0	0 ± 0	25 ± 1.0

Inhibition of biofilm formation

The ability of the herbal products to *in vitro* inhibit pathogenic biofilm establishment was appraised using the microtiter plate with crystal violet staining for semiquantitative assessment. Crystal violet-stained wells are represented in figure 2 and a statistical comparison of the semi-quantified biofilm biomass is illustrated in figure 3. Table 3 depicts the percentage of inhibition of the treated biofilm to the untreated control. *S. aureus*, *P. aeruginosa* and *C. albicans* biofilms were the densest when compared to biofilms formed by *E. coli* and *E. faecium* (Figures 2 and 3). However, the biomasses of the dense biofilms of the former pathogens significantly declined when co-cultured with the four herbal products tested. Renalka™ was most effective ($p < 0.001$) in suppressing *P. aeruginosa* biofilm formation (% inhibition = 97.8 %) while Cystone® was the least effective (% inhibition = 74.0%), though still significant ($p < 0.05$). *S. aureus* biofilm formation was kerbed the most (% inhibition = 90.6%) when cultured with UROCLEAR ($p < 0.001$) and suppressed the least (% inhibition = 57.8%) in response to ROWAtinex® ($p < 0.05$). Semi-quantitative assessment of *C. albicans* biofilm biomass demonstrated the most significant ($p < 0.005$) growth restriction (% inhibition = 94.4%) when the yeast was mixed with Renalka™ (% inhibition = 94.4%). Conversely, Cystone® and UROCLEAR were found able to enhance the estimated *E. coli* biofilm biomass while Renalka™ was shown effective ($p < 0.05$) in suppressing the formation of the enteric coli biofilm (% inhibition = 53.5%). *E. faecium* biofilm biomass was also diminished significantly by co-culturing with Cystone® with a percentage of inhibition of 90.7 % ($p < 0.005$) whereas ROWAtinex® reduced the biomass by 38.9% but non-significantly ($p > 0.05$).

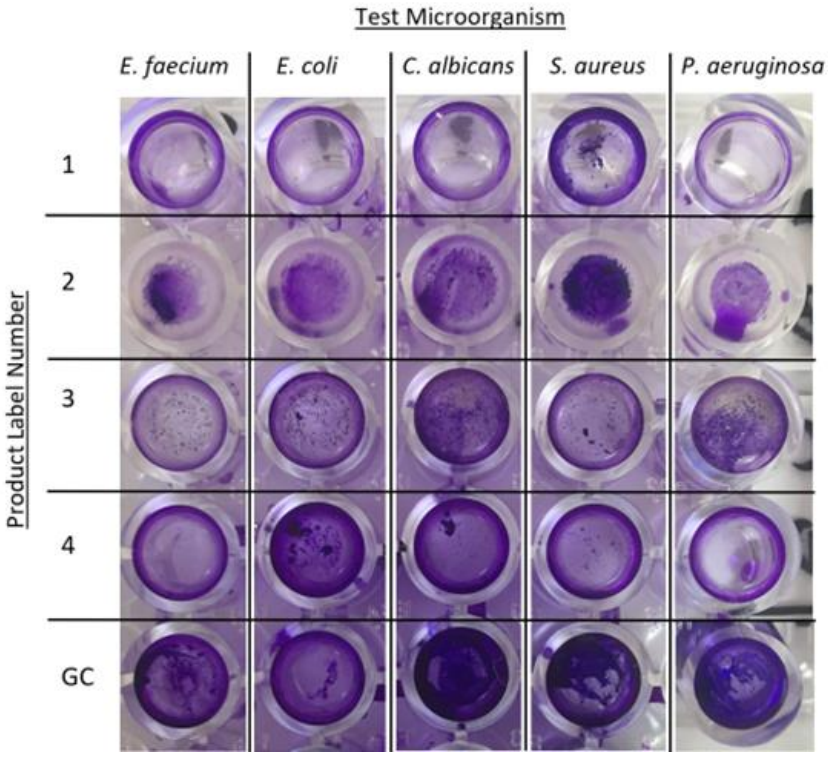

Figure 2. Antibiofilm activity of the herbal products against bacterial and yeast biofilm formation. Prevention of biofilm attachment was assessed by crystal violet assay. 1: Renalka™ , 2: ROWAtinex®, 3: Cystone®, 4: UROCLEAR, GC: growth control.

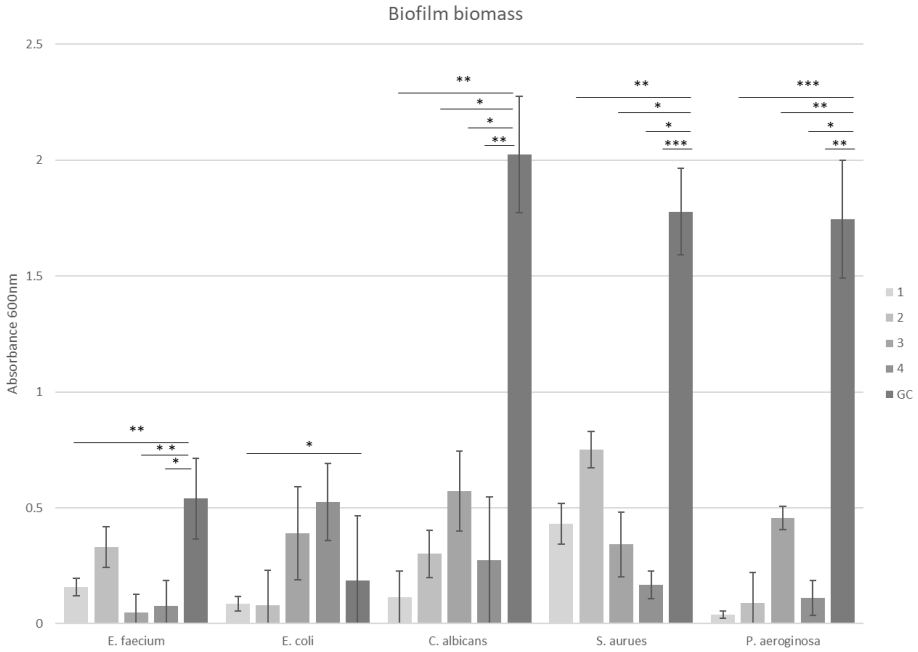

Figure 3. The repressive effects of the herbal products on bacterial and fungal biofilm formation. Semi-quantitative assessment by crystal violet staining of the biofilm biomass. 1: Renalka ™ , 2: ROWAtinex ®, 3: Cystone ®, 4: UROCLEAR, GC: growth control *; $p > 0.05$, **; $p > 0.005$, ***; $p > 0.001$ using Dunnett's one way ANOVA.

Table 3. Percentage of inhibition of biofilm biomass

Test Microorganism	**Herbal Product**			
	Renalka™	**ROWAtinex®**	**Cystone®**	**UROCLEAR**
P. aeruginosa	97.8	95.0	74.0	93.6
S. aureus	75.8	57.8	80.8	90.6
C. albicans	94.4	85.1	71.8	86.5
E. coli	53.5	57.8	0	0
E. faecium	70.7	38.9	90.7	85.9

DISCUSSION

Many polyherbal formulations are extensively described for cases of urinary

disorders, however, no *in situ* studies have evaluated theirs *in vitro* antimicrobial and antibiofilm potentials against pathogenic clinical isolates. Four commercial herbal products available in the local market indicated for treating urinary tract disorders such as urolithiasis, renal stones, and urinary tract infections were assessed for *in vitro* microbial growth inhibitory and antibiofilm effect. Five selected uropathogenic microbes were included. *In vitro*, well diffusion assessment demonstrated antimicrobial activity of Renalka™, ROWAtinex®, and UROCLEAR against *C. albicans*, *S. aureus,* and *E. faecium*. A significant antibiofilm activity was reported for all of the tested products against the pathogenic biofilm formation but to a varying degree.

These polyherbal products have been used for a variety of symptoms relieving, prophylactic and therapeutic purposes. Starting with Renalka™, it has been extensively studied for its urinary beneficial effect (7). Currently, it was shown effective to only inhibit the growth of *E. faecium*, however dramatic inhibition of biofilm formation was encountered against all tested pathogens in response to the co-culture with Renalka™. The beneficial health effect could be due to one or more of the active herbal constituents. The principal element of Renalka™ ; Gokshura (*Tribulus terrestris*) has been shown to effectively enhance renal function via a number of mechanisms including induction of diuresis, increasing the rate of glomerular filtration and chloride and creatinine clearance (13). It also revealed analgesic and anti-inflammatory action (6). Regarding antimicrobial activity, and in accordance with our finding, a study conducted by Ali et al. (14) demonstrated no antimicrobial effect of gokshura against *S. aureus, E. coli* and *P. aeruginosa*. However, a strong recommendation of using gokshura as a quorum quenching agent (antibiofilm agent) against *P. aeruginosa* was obtained from a recent study published in 2019 (15). *Cretaeva magna* extract was reported to have a good antimicrobial activity against *S. aureus* and *E. coli* (16). However, no antimicrobial effect against a number of pathogenic bacteria and fungi was documented by Farjana et al. (17). Concerning its antibiofilm potential and up to the best of our knowledge, no information was retrieved from the published research articles. The root bark extract of Hemidesmus indicus (18) was shown effective in inhibiting *S. aureus* biofilm formation. Another study demonstrated a powerful antibiofilm effect of Hemidesmus indicus against clinical, multi-drug resistance strains of *E. coli*, *P. aeruginosa*, *S. aureus,* and *Bacillus subtilis* (19). Cyperus rotundus has been informed to exert antimicrobial activity against a number of pathogenic microbes including *C. albicans* (20,21) while its antibiofilm effect was reported against *Actinobacteria baumannii* (22). Antibiofilm potential against pathogenic *S. aureus* isolates was documented on behalf of Vetiveria zizanioides root extract (23) in addition to antimicrobial effect. Asparagus is also reported to exert antibiofilm effect against *Candida* species (24). Trikatu is an important primeval formula composed of black pepper with long pepper and ginger. The therapeutic effect has been attributed mainly to the active ingredient piperine which has recently demonstrated a valuable antibiofilm effect against the pathogenic yeast *C. albicans* (25). Similarly, Elletaria has been tested for antibiofilm effect against Gram-negative pathogens and results demonstrated a beneficial effect (26).

ROWAtinex® revealed an antimicrobial effect against *S. aureus* and *E. faecium* in the present study. Another study conducted to sightsee the antimicrobial action of Rowatinex® demonstrated a lack of any inhibitory effect of urine samples taken from volunteers consuming

Rowatinex® on the growth of common pathogens of urinary system. However, an antimicrobial effect was encountered *in vitro* indicating that the metabolized forms of the product excreted in the urine retained no antibacterial effect (27). α-pinene and β-pinene, two major constituents of Rowatinex®, were studied for antimicrobial and antibiofilm effects against *S. aureus* and came up with excellent antimicrobial and antibiofilm effects (28). Camphene was also documented to have an antimicrobial, antibiofilm and antivirulence potentials against Gram-positive and Gram-negative bacteria (29). Borneol had also antibacterial effects (30) and antibiofilm with an antihyphal effect against *C. albicans* (31). In addition, anethole was shown to exert antimicrobial and antibiofilm potentials (Kubo et al. 2008). *C. albicans* antibiofilm effect was also reported for fenchone (31). Moreover, uropathogenic biofilms were shown to be inhibited by cineol (32).

Cystone® did not show an antimicrobial effect against any of the tested pathogens but had antibiofilm formation activity except for *E. coli* where it oppositely enhanced biofilm formation. Contrary to the present finding, Rubia cordifolia was shown to exhibit antistaphylococcal effect (33) which is one of the components of cystone®. Silver nanoparticles unravelled by means of utilizing root and stem extracts from *Rubia cordifolia* were tested for antimicrobial and antibiofilm effects and demonstrated the aptitude to impede the growth and biofilm formation of a number of human pathogens including uropathogens (34).

At present, UROCLEAR was shown effective in inhibiting biofilm formation of the tested pathogens except for *E. coli*. Similarly, Ulrey et al. (35) found antibiofilm and anti-swarming trend of cranberry extract (the main constituent of UROCLEAR) against *P. aeruginosa* in a dose-dependent style. Contrary to our findings, a number of studies have reported an effective antibiofilm effect of cranberry against uropathogenic *E. coli* (36,37,38). The deficiency of biofilm inhibitory activity against *E. coli* encountered in the current study might be attributed to the virulence of the pathogenic strain studied and the small sample size. A meta-analysis review study demonstrated the apparent effectiveness of D-mannose for recurrent urinary tract infections with activity comparable to antibiotics with minimal side effects (39). Mannose also demonstrated the ability to disrupt *P. aeruginosa* biofilm formation *in vitro* (40).

CONCLUSION

The findings of the current study fortify the ethnobotanical value of the studied herbal products with their bioactive ingredients for prophylactic use against uropathogenic biofilm formation in addition to their indication for different renal system disorders.

ACKNOWLEDGEMENT

The author is appreciative of the Pharmacy College/Mosul University for supplying the needed facilities to run this research.

CONFLICT OF INTEREST

The author has declared that there is no a conflict of interest for this study.

REFERENCES

1- Mamtani R, Cheema S, MacRae B, Alrouh H, Lopez T, ElHajj M and Mahfoud Z. 2015. Herbal and nutritional supplement use among college students in Qatar. East Mediterr Health Journal, 21(1), pp.39-44.

2- WHO. Global report on traditional and complementary medicine 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO

3- Kunin C. 1987. Urinary Tract Infections Detection, prevention and management. 5th ed. Baltimore, MD: Willams & Wilkins.
4- Ronald AR, Nicolle LE, Stamm E, Krieger J, Warren J, Schaeffer A, Naber KG, Hooton TM, Johnson J, Chambers S and Andriole V. 2001. Urinary tract infection in adults: research priorities and strategies. International Journal of Antimicrobial Agents, 17(4), pp.343-348.
5- Stamm WE, McKevitt M, Roberts PL and White NJ. 1991. Natural history of recurrent urinary tract infections in women. Reviews of Infectious Diseases, 13(1), pp.77-84.
6- Bazzaz BSF, Fork SD, Ahmadi R and Khameneh B. 2021. Deep insights into urinary tract infections and effective natural remedies. African Journal of Urology, 27(1), pp.1-13.
7- Desai MG and Palaniyamma D. 2012. A Clinical Review on a Polyherbal Formulation, Renalka Syrup in Urinary Tract Infection. Indian Journal of Clinical Practice, 22(12): 615-620.
8- Qasim ZS. 2020. The Effect of Ginkgo biloba Extracts on Candida albicans Isolated from Healthy Persons. Iraqi Journal of Pharmaceutical Sciences, 29(2), pp.122-128.
9- Vidyashankar S, Maheshkumar P and Patki PS. 2010. Cystone–An ayurvedic polyherbal formulation inhibits adherence of uropathogenic *E. coli* and modulates H2O2-induced toxicity in NRK-52E cells. Journal of Experimental Pharmacology, 2, p.19.
10- Hashim ZA. 2022. Evaluation of bacterial contents, package labelling and antimicrobial activity of some commercial probiotic products available in local market. Journal of Research in Pharmacy, 26(3), pp.502-509.
11- Sandasi M, Leonard CM, Viljoen AM. 2010. The *in vitro* antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. Letters in Applied Microbiology, 50:30–5.
12- Shinde S, Lee LH, Chu T. 2021. Inhibition of Biofilm Formation by the Synergistic Action of EGCG-S and Antibiotics. Antibiotics (Basel), 21;10(2):102.
13- Al-Ali M, Wahbi S, Twaij H, Al-Badr A. 2003. *Tribulus terrestris*: preliminary study of its diuretic and contractile effects and comparison with Zea mays. Journal of Ethnopharmacology, 85(2-3):257-60.
14- Ali NA, Jülich WD, Kusnick C, Lindequist U. 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology, 74(2):173-9.
15- Vadakkan K, Vijayanand S, Hemapriya J and Gunasekaran R. 2019. Quorum sensing inimical activity of *Tribulus terrestris* against gram negative bacterial pathogens by signalling interference. Biotechnology, 9(4), pp.1-6.
16- Meera R and Venkataraman S. 2017. Phyto chemical screening and anti Microbial activity of root extract of Crataeva magna Lour (DC). Research Journal of Pharmacy and Technology, 10(8), pp.2657-2662.
17- Farjana, A., Zerin, N., &Kabir, M.d. S. (2014). Antimicrobial activity of medicinal plant leaf extracts against pathogenic Bacteria. Asian Pac J Trop Dis, 4(2): 920-923.
18- Kannappan A, Santhakumari S, Srinivasan R, Pandian SK, Ravi AV. Hemidesmus indicus, a traditional medicinal plant, targets the adherence of multidrug-resistant pathogens to form biofilms. Biocatalysis and Agricultural Biotechnology. 2019 Sep 1;21:101338.
19- Girish N and Mamatha A. 2020. Bacterial biofilm inhibition activity of ethanolic extract of Hemidesmus indicus. Indian Journal of Traditional Knowledge, 19(3), pp.652-655.
20- Najah AM. 2012. *In vitro* inhibitory effect of Cyperus rotundus L crude extracts on mouth isolates of *Streptococcus mutans* and *Candida*

albicans. Al Mustansiriyah Journal of Pharmaceutical Sciences, 11(1), pp.85-91.
21- Yu HH, Lee DH, Seo SJ and You YO. 2007. Anticariogenic properties of the extract of Cyperus rotundus. The American Journal of Chinese Medicine, 35(03), pp.497-505.
22- Selvaraj A, Valliammai A, Sivasankar C, Suba M, Sakthivel G, Pandian SK. Antibiofilm and antivirulence efficacy of myrtenol enhances the antibiotic susceptibility of Acinetobacter baumannii. Scientific reports. 2020 Dec 15;10(1):1-4.
23- Kannappan A, Gowrishankar S, Srinivasan R, Pandian SK, Ravi AV. 2017. Antibiofilm activity of Vetiveria zizanioides root extract against methicillin-resistant *Staphylococcus aureus*. Microbial Pathogenesis, 110:313-324.
24- Jayaraman L, Sellamuthu PS, Siva DP, Aravindan RS, Vellaichamy V and Anitha PM. 2021. *In Vitro* Antibacterial, Antioxidant and Mechanism of Antifungal Activities of Selected Indian Traditional Plants against Human Oral Microbes. Indian Journal of Pharmaceutical Sciences, 83(5), pp.1024-1032.
25- Priya A, and Pandian SK. 2020. Piperine impedes biofilm formation and hyphal morphogenesis of *Candida albicans*. Frontiers in Microbiology, 11, p.756.
26- Asghar A, Algburi A, Huang Q, Ahmad T, Zhong H, Javed HU, Ermakov AM and Chikindas ML. 2021. Anti-biofilm potential of Elletaria cardamomum essential oil against *Escherichia coli* O157: H7 and *Salmonella Typhimurium* JSG 1748. Frontiers in Microbiology, 12, p.749.
27- Greenwood D, Johnson N, Eley A, Slack RCB and Bell GD. 1982. The antibacterial activity of Rowatinex. Journal of Antimicrobial Chemotherapy, 10(6), pp.549-551.
28- Rivas da Silva AC, Lopes PM, Barros de Azevedo MM, Costa DC, Alviano CS and Alviano DS. 2012. Biological activities of α-pinene and β-pinene enantiomers. Molecules (Basel, Switzerland), 17(6), pp.6305-6316.
29- Adeosun IJ, Baloyi IT and Cosa S. 2022. Anti-Biofilm and Associated Anti-Virulence Activities of Selected Phytochemical Compounds against *Klebsiella pneumoniae*. Plants, 11(11), p.1429.
30- Tabanca N, Kırımer N, Demirci B, Demirci F and Başer KHC. 2001. Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phrygia* and the enantiomeric distribution of borneol. Journal of Agricultural and Food chemistry, 49(9), pp.4300-4303.
31- Manoharan RK, Lee JH and Lee J. 2017. Antibiofilm and antihyphal activities of cedar leaf essential oil, camphor, and fenchone derivatives against *Candida albicans*. Frontiers in Microbiology, 8, p.1476.
32- Lagha R, Ben Abdallah F, Al-Sarhan BO and Al-Sodany Y. 2019. Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against *Escherichia coli* isolated from UTI patients. Molecules, 24(6), p.1161.
33- Watroly MN, Sekar M, Fuloria S, Gan SH, Jeyabalan S, Wu YS, Subramaniyan V, Sathasivam KV, Ravi S, Rani IM and Lum PT. 2021. Chemistry, biosynthesis, physicochemical and biological properties of rubiadin: A promising natural anthraquinone for new drug discovery and development. Drug Design, Development and Therapy, 15, p.4527.
34- Mariswamy SD, Thimmaiah CK, Basappachidananda VK, Basavaraju M and Arkeswaraiah CN. 2021. Antimicrobial, haemolytic and antibiofilm assay of green synthesized silver nanoparticles by aqueous extracts of Rubia cordifolia. International Journal of Pharmaceutical Sciences Review and Research, 28, pp.174-184.
35- Ulrey RK, Barksdale SM, Zhou W and van Hoek ML. 2014. Cranberry

proanthocyanidins have anti-biofilm properties against *Pseudomonas aeruginosa*. BMC Complementary and Alternative Medicine, 14(1), pp.1-12.

36- Tapiainen T, Jauhiainen H, Jaakola L, Salo J, Sevander J, Ikäheimo I, Pirttilä AM, Hohtola A and Uhari M. 2012. Biofilm formation and virulence of uropathogenic *Escherichia coli* in urine after consumption of cranberry-lingonberry juice. European Journal of Clinical Microbiology & Infectious Diseases, 31(5), pp.655-662.

37- Yamanaka A, Kimizuka R, Kato T and Okuda K. 2004. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiology and Immunology, 19(3), pp.150-154.

38- Yamanaka A, Kouchi T, Kasai K, Kato T, Ishihara K and Okuda K. 2007. Inhibitory effect of cranberry polyphenol on biofilm formation and cysteine proteases of *Porphyromonas gingivalis*. Journal of Periodontal Research, 42(6), pp.589-592.

39- Lenger SM, Bradley MS, Thomas DA, Bertolet MH, Lowder JL and Sutcliffe S. 2020. D-mannose vs other agents for recurrent urinary tract infection prevention in adult women: a systematic review and meta-analysis. American journal of obstetrics and gynecology, 223(2), pp.265-e1.

40- Limqueco E, Passos Da Silva D, Reichhardt C, Su FY, Das D, Chen J, Srinivasan S, Convertine A, Skerrett SJ, Parsek MR and Stayton PS. 2020. Mannose conjugated polymer targeting *P. aeruginosa* biofilms. ACS Infectious Diseases, 6(11), pp.2866-2871.