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Phytochemical and Antibacterial Activity of *Aspilia africana* Extract on Bacterial Isolates from Patients with Urinary Tract Infection in Yenagoa Metropolis

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Abstract

The phytochemical and antibacterial properties of Aspilia africana extracted via ethanol, petroleum ether, acetone and aqueous solvents were investigated in this study. Fifty urine specimens were obtained from patients diagnosed with urinary tract infection and were studied by culture, gram staining and biochemical analysis. Susceptibility testing and the minimum inhibition concentration (MIC) of Aspilia africana extracts were ascertained with the agar dilution method. Data obtained were analysed with the analysis of variance (ANOVA) statistical test. Thirty-nine of the isolates were identified as Escherichia coli 14(35.9%), Staphylococcus aureus 19(48.7%), Klebsiella spp. 3(7.7%) and Pseudomonas aeruginosa 3(7.7%). The distribution of bacteria by sex recorded female with highest occurrence of 12(30.8%) while male with the least 1(2.6%). The distribution of bacterial isolates by sex showed significant difference ($P < 0.05$). The age interval of 24-29 years had the highest occurrence 11(28.2%) while 36-41 years had the least with 6(15.4%). The distribution of bacterial isolates by age were significantly different ($P < 0.05$). Qualitative phytochemical analysis identified the presence of flavonoids, tannins, alkaloids, saponins and carbohydrate in the investigated plant. Quantitative phytochemical evaluation of plant extracts revealed phenols (0.643±0.006), tannins (0.222±0.008), anthocyanin (1.27±0.03), flavonols (17.52±2.11) and flavonoids (20.42±1.45). The ethanol extracts of Aspilia africana showed significant inhibitory action ($p<0.05$) against the bacterial isolates at 250 mg/ml. The highest mean diameter zone of inhibition for all the extracts was 15 mm at 250 mg/ml for E. coli while the least 0.7 mm at 31.25 mg/ml for Pseudomonas aeruginosa. The MIC of all extracts against the isolates were observed at 31.25 mg/ml. Therefore, results obtained reveal that Aspilia africana holds potentials for novel drugs development for treatments of bacterial infections.

1. Introduction

It is an aged practice in most parts of the world, that unique medicinal plants are utilised for treatment and managements of different infectious diseases. The World Health Organization (WHO) estimates that 80% of the world population use herbal regimen for treatment and control of

diseases [1]. The need to establish therapeutic efficacy of herbs has triggered scientific investigations of several medicinal plants including the evaluation of their antibacterial potencies. Despite, the high cost of some antibiotics, many commonly used antibiotics have serious setbacks. "A good number of standard antibiotics have been found to be neurotoxic, nephrotoxic and hypertensive, and few others cause severe damage to liver and bone marrow depression" [2]. The main benefit of plant derived medicine is that they are comparatively safer and less expensive than synthetic antibiotics, and they offer profound therapeutic benefits [3]. Medicinal plants are continuously investigated in order to provide an alternative to conventional antibiotics, which will reduce cost and side effects. Multi-drug resistance (MDR) is one factor that limits the use of cheap and old antibiotics. MDR has prompted the increase in research for development of new modified antimicrobial agents of herbal origin to combat resistance [4]. Many medicinal plants possess the ability to produce intrinsic antimicrobial compounds and MDR inhibitors, which increases antibacterial activities especially on antibiotic-resistant pathogenic bacteria [5, 6, 7].

Urinary tract infection (UTI) refers to bacterial infections that affect all sections of the urinary tract. Cystitis pertains to UTI that affects the lower urinary tract and involves the bladder, while pyelonephritis is a type of UTI that generally affects the upper urinary tract and involves the kidneys. Symptoms of cystitis include painful and frequent urination and the heightened urge to urinate despite an empty bladder. For cases of pyelonephritis, symptoms range from fever, flank pain, painful and frequent urination and the increased urge to urinate even with an empty bladder. There is hardly an evidence of blood in urine with suspected UTI cases. UTI affects individuals of all ages, however symptoms of UTI are not definitive or specific and can be clinically misconstrued [8, 9].

About 150 million people are affected with UTI yearly, with women been most affected (10). The common causative organism of UTI is the *Escherichia coli*, [11], however other organism such fungi could be involved, although rarely. Risk factors associated with UTI include the female anatomy, sexual intercourse, and underlying condition such as diabetes; obesity, and family history. Despite sexual intercourse been referred to as a risk factor, UTIs are not considered as sexually transmitted infections [12]. Diagnosis of UTI is solely based on mostly symptoms when diagnosing young healthy women [13], however in women with unclear symptoms, diagnosis may not be easy because the presence of bacteria in the urinary tract has not led to infections [14, 15]. In the treatment of UTI, antibiotic resistance is on the increase. When UTI cases are uncomplicated, the antibiotic nitrofurantoin is well sought after and taken as a short-course treatment [16, 17] or the antibiotic, trimethoprim/sulfamethoxazole is used as an alternative [18]. In complicated cases or treatment failure, a urine culture is useful to ascertain diagnosis [19, 20] and a longer duration or intravenous antibiotics may be needed [21]. In the eventuality that there is no improvement of symptoms, further diagnostic testing would be necessary. Phenazopyridine is usually administered to help with symptoms. Due to the demand for an innovative and ideal antibiotic that may substitute or supplement existing regimens, this study is set to provide results necessary to combat MDR. This investigation will evaluate the antibacterial activities of *Aspilia africana* on bacteria obtained from patients with UTI.

2.0 Methodology

2.1 Specimen Collection, Isolation and Identification

Fifty urine specimens were obtained from patients attending Scanex Diagnostics Yenagoa Metropolis, Bayelsa State. Specimens were cultured on Mannitol salt agar, MacConkey agar and Eosin Methylene Blue agar respectively. The agar plates were incubated at 37^{o}C for 24 hours and examined for growth. Pure isolates were characterized using gram staining technique and biochemical tests.

2.1.1. Collection and Identification of Plant Material

The plant (*Aspilia Africana)* was obtained from a farmland at Azikoro in Yenagoa, Bayelsa State

Nigeria. They were authenticated by with voucher specimen number MP-196 from Pharmacognosy Department, Madonna University, Nigeria, Elele, Rivers State, Nigeria.

2.2. Preparation of Plant Extract

The leaves of *Aspilia africana* was plucked and rinsed with water to remove dirt. They were dried at room temperature for four (4) weeks. The dried leaves were ground into fine powder. The active ingredients of the plant were extracted by soxhlet extraction method using petroleum ether, ethanol, acetone and water. 300 ml of each solvent were used for the extraction. 30 g of the crushed plant material was wrapped with a filter paper; and loaded into the thimble jacket, placed on the round bottom flask containing the solvent. The solvent was heated and evaporated with a rotary evaporator, while a small yield of the extracted plant material left in the glass bottom flask. The dried crude extracts were then transferred into sterile air-tight containers and stored in the refrigerator (4ºC) for subsequent use (22). The stored extracts were liquefied in appropriate solvents for respective phytochemical and antibacterial assay.

2.3. Phytochemical screening

This was done using qualitative phytochemical analysis and quantitative phytochemical analysis

2.4 Qualitative Phytochemical Analysis

Test for flavonoids, tannins, carbohydrate, glycosides, saponins, resins, terpenoids and alkaloids were carried out using standard methods [23, 24, 25].

2.5 Quantitative Phytochemical Analysis: This involves the determination of the total phenols, tannin, total flavonoids and total anthocyanin contents.

Determination of total phenols was done using Folin-Ciocalteau reagent (FCR) as described by [26] with slight modifications.

Tannin content was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by [27].

The flavonoids content was determined using the method described by [28] with slight modifications.

The total anthocyanin contents of the plant extracts were measured using a spectrophotometric pH differential protocol as described by [29] and [30] with slight modifications.

2.6 Antibacterial assay

One gram of each crude extract was dissolved in 4 ml of 20% dimethylsulfoxide (DMSO) to obtain a concentration of 250 mg/ml of the extract. This was serially diluted in two folds to obtain the following extract concentrations: 250, 125, 62.5 and 31.25 mg/ml. The antibacterial activities of the plant extracts on isolates were obtained by using agar well dilution method [31, 32, 33]. An eighteen hour Mueller Hinton broth cultures containing the bacterial isolates were diluted and standardized to 0.5 McFarland turbidity standard using sterile normal saline. The standardized broth culture was evenly inoculated on the Mueller Hinton agar by streaking. The inoculated Mueller Hinton agar was for about 5 minutes to dry, afterwards wells were punched on the agar at equidistant positions using a sterile standard 6mm cork borer. About 60 µl of different concentrations of the extract were respectively introduced into the different wells that have been labeled. In addition, with the use of a micropipette, about 60 µl of the 20% DMSO was introduced into the well bored in the centre of the agar medium as a control. This procedure was done in duplicate for all the bacterial isolates and left for 30 minutes before a 24 hour incubation at 37^0C. Following the incubation, the inhibition zone diameters produced by the different concentrations of the crude extracts were measured. Antibacterial activities determined were expressed as mean inhibition zone diameters (mm) in the duplicate experiment.

2.7. Determining the Minimum Inhibitory Concentration (MIC) of Plant Extracts

Minimal inhibitory concentrations of the extracts on the bacterial isolates were determined by macro broth dilution method recommended by Clinical and Laboratory Standard Institute [34].

One gram of the extract was dissolved in 1ml of 20% DMSO to obtain an extract concentration of 250mg/ml. Various serial dilutions were made from this stock solution in tubes of 1 ml sterile Mueller Hinton broths to get 125mg/ml, 62.5 mg/ml, and 31.25mg/ml. An overnight nutrient broth culture of the test bacterial isolate was adjusted to 0.5 McFarland turbidity standards. Different dilutions of the suspension were made in a sterile normal saline to obtain a final inoculum concentration of 106 CFU/ml. Then 1 ml of this adjusted inoculum was put into each tube of the Mueller Hinton broth containing different concentration of the crude extract. Each of the tubes were mixed and then incubated at 37^0C for 24hours [33]. This experiment was done twice for all the bacterial isolates. Mueller Hinton broth containing 1ml suspension of the isolate in a tube without extract and tubes of Mueller Hinton broth with different concentrations of the extract without the isolate were used as controls. The tubes were examined after 24hrs incubation. The MIC of the extract was taken as the lowest extract concentration that has no growth of the bacterial isolates in the tubes, as indicated by lack of visual turbidity.

3.0 Results

Out of the fifty (50) specimens collected from patients at the Scanex Diagnostics, a total of thirty-nine bacteria isolates were successfully obtained. Bacterial species isolated include *Escherichia coli* 14 (35.9%), *Staphylococcus aureus* 19 (48.7%), *Klebsiella* spp 3 (7.7%) and *Pseudomonas aeruginosa* 3 (7.7%).

Table 1 shows the frequency distribution of bacterial isolates from patient with UTI in Yenagoa Metropolis by age. *Staphylococcus aureus* had the highest occurrence of 19 (48.7%) while the lowest was *Klebsiella* spp and *Pseudomonas aeruginosa* with 3 (7.7%) respectively.

Table 2 shows the distribution of bacterial isolate among patients with UTI in Yenagoa Metropolis by sex. *Staphylococcus aureus* was observed with the highest frequency of 12(30.8%) in female while the least was *Klebsiella* spp and *Pseudomonas aeruginosa* with 1(2.6%) respectively in male.

Table 3 shows the diameter zone of inhibition (mm) of acetone extract of *Aspilia africana* on bacterial isolates from UTI. The highest diameter for the zone of inhibition was 14 mm at 250 mg/ml concentration against *Klebsiella* spp while the least 0.7 mm at 31.25 mg/ml concentration against *Pseudomonas aeruginosa*. Significant difference ($p > 0.05$) in the inhibition with different concentration of acetone extract was observed.

Table 4 shows the diameter zone of inhibition (mm) of petroleum ether extract of *Aspilia africana* on bacterial isolates from UTI. The highest diameter zone of inhibition was 12.7 mm at 125 mg/ml concentration against *Klebsiella* spp while the least 3.3 mm at 250 mg/ml concentration against *Pseudomonas aeruginosa* as seen in Table 4.

Table 5 shows the diameter zone of inhibition (mm) of ethanol extract of *Aspilia africana* on bacterial isolates from UTI. The highest diameter zone of inhibition observed was 15 mm at 250 mg/ml concentration against *Escherichia coli* while the least 1.7 mm at 31.25 mg/ml concentration against *Staphylococcus aureus*. A significance ($p > 0.05$) was observed in the inhibition in different concentration for ethanol extract as seen in Table 5.

Table 6 shows the diameter zone of inhibition (mm) of aqueous extract of *Aspilia africana* on bacterial isolates from UTI. The highest diameter zone of inhibition seen was 13 mm at 250 mg/ml concentration against *Klebsiella* spp while the least 1.7 mm at 62.5 mg/ml concentration against *Pseudomonas aeruginosa*.

Table 7 shows the minimum inhibitory concentration (MIC) values of extract of *Aspilia africana* against bacterial isolates. The MIC values of the extracts against the isolates were obtained from the agar diffusion assay. The lowest MIC for *E.coli* was obtained with ethanol extract; *S. aureus,* with

Petroleum ether and ethanol extracts; *Klebsiella* spp with acetone, ethanol and aqueous extracts; and *Pseudomonas* spp with acetone extract.

Table 8 shows the qualitative phytochemical constituent of *Aspilia africana* which revealed the presence of important phytochemical constituents. flavonoids, tannins, alkaloids saponins and carbohydrates were the major phytochemical constituents present in the extracts in relative abundance.
Table 9 shows the quantitative phytochemical constituents *Aspilia africana*. The amount of total phenolics was 0.643±0.006 mgGAE/mg of dry plant extracts of *Aspilia africana*. The total flavonoid content was 20.42 ±1.45 rutin equivalents/g dry weight plant extract of *Aspilia africana*.

Table 1: Percentage distribution of bacterial isolates from patents with UTI in Yenagoa Metropolis by age.

Age Interval	*Escherichia coli* (%)	*Staphylococcus aureus* (%)	*Klebsiella* species (%)	*Pseudomonas aeruginosa* (%)	Total
18 - 23	3 (7.7)	3 (7.7)	1 (2.6)	0 (0)	7 (17.9)
24 - 29	5 (12.8)	5 (12.8)	0 (0)	1 (2.6)	11 (28.2)
30 - 35	3 (7.7)	4 (10.3)	1 (2.6)	0 (0)	8 (20.5)
36 - 41	2 (5.1)	2 (5.1)	1 (2.6)	1 (2.6)	6 (15.4)
42 - 47	1 (2.6)	5 (12.8)	0 (0)	1 (2.6)	7 (17.9)
Total	14 (35.9)	19 (48.7)	3 (7.7)	3 (7.7)	39 (100)

Table 2: Percentage distribution of bacterial isolates from patents with UTI in Yenagoa Metropolis by sex.

Isolate	No of Isolate	Sex		
		Male (%)	Female (%)	Total
Escherichia coli	14	8(20.5)	6(15.4)	14(35.9)
Staphylococcus aureus	19	7(17.9)	12(30.8)	19(48.7)
Klebsiella spp	3	1(2.6)	2(5.1)	3(7.7)
Psuedomonas aeruginosa	3	1(2.6)	2(5.1)	3(7.7)
Total	39	17(43.6)	22(56.4)	39(100)

Table 3: Diameter zone of inhibition (mm) of acetone extract of *Aspilia africana* on bacterial isolates from UTI

Organisms	Concentrations			
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
Escherichia coli	3.5	2.3	2.3	-
Staphylococcus aureus	6.5	5.3	1.3	-
Klebsiella spp	14.0	12.0	6.0	5.3
Pseudomonas aeruginosa	9.3	5.3	2.0	0.7

Data represented as Mean ± SD (n = 3)
Key:
mg/ml = milligram per milliliter
- = No zone of inhibition

Table 4: Diameter zone of inhibition (mm) of Petroleum ether extract of *Aspilia africana* on bacterial isolates from UTI

Organisms	Concentrations			
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
Escherichia coli	7.0	-	-	-
Staphylococcus aureus	11.0	6.7	8.0	5.7
Klebsiella spp	8.7	12.7	6.0	-
Pseudomonas aeruginosa	3.3	-	-	-

Data represented as Mean ± SD (n = 3)

Key:

mg/ml = milligram per milliliter

- = No zone of inhibition

Table 5: Diameter zone of inhibition (mm) of ethanol extract of *Aspilia africana* on bacterial isolates from UTI

Organisms	Concentrations			
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
Escherichia coli	15.0	10.3	7.0	3.0
Staphylococcus aureus	13.0	10.3	6.3	1.7
Klebsiella spp.	11.3	8.7	7.0	3.3
Pseudomonas aeruginosa	11.3	8.7	5.3	-

Data represented as Mean ± SD (n = 3)

Key:

mg/ml = milligram per milliliter

- = No zone of inhibition

Table 6: Diameter Zone of inhibition (mm) of aqueous extract of *Aspilia africana* on bacterial isolates from UTI

Organisms	Concentrations			
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
Escherichia coli	6.0	4.0	3.0	-
Staphylococcus aureus	12.0	8.0	2.7	-
Klebsiella spp	13.0	10.3	8.3	5.7
Pseudomonas aeruginosa	8.0	4.7	1.7	-

Data represented as Mean ± SD (n = 3)

Key:

mg/ml = milligram per milliliter

- = No zone of inhibition

Table 7: Minimum Inhibitory Concentration (MIC) Values of Extract of *Aspilia africana* against bacterial isolates

Bacterial isolates	Minimum Inhibition Concentration (mg/ml)			
	Acetone	Petroleum ether	Ethanol	Aqueous
E. coli	-	-	31.25	-
Staphylococcus aureus	-	31.25	-	-
Klebsiella spp	31.25	-	31.25	31.25
Pseudomonas aeruginosa	31.25	-	-	-

Key: mg/ml = milligram per milliliter
- = no MIC value obtained

Table 8: Qualitative Phytochemical Constituents of *Aspilia africana*

Plant Constituents	*Aspilia Africana*
Flavonoids	+++
Tannins	++
Alkaloids	++
Terpenoid	-
Glycoside	+
Saponins	++
Carbohydrate	++
Resin	-
Protein	+

Key:
+ = Present ++ = Moderately present
+++ = Abundant - = Absent

Table 9: Quantitative Phytochemical Constituents of *Aspilia africana*

Extract	**Phenolic contents ***			**Total anthocyanin †**	**Total flavonols ‡**	**Total flavonoids ‡**
	Total Phenols	**Non-tannins**	**Tannins**			
Aspilia africana	0.643± 0.006	0.478 ± 0.031	0.222 ± 0.008	1.27 ± 0.03	17.52 ± 2.11	20.42 ±1.45

Data represented as Mean ± SD (n = 3)
* Expressed as mg gallic acid equivalents (GAE) / mg dry weight plant extract
† Expressed as mg cyanidin 3-glucoside equivalents (CGE) /100g of dry weight extract
‡ Expressed as mg rutin equivalents (RE) / g dry weight plant extract

3.1 Discussion

The existence and involvement of bacteria in UTI cannot be overlooked as the increasing incidence of UTI has become a health issue globally. Despite the common use of antibiotics in the treatment of this infection, preference for medicinal plant cannot be over emphasized. The qualitative analysis carried out indicate the presence of important phytochemical constituents (Table 8). *Aspilia africana* contained a mixture of polyphenolic compounds such as tannins, flavonoids, alkaloids, glycosides and protein. Quantitative phytochemical screening also revealed high phenolic contents. The high

recovery of the phytochemical constituents, the flavonoids in particular in this study corroborated with findings of [35]. The putative antibacterial potential of this plant extract could be attributed therefore, to the presence of some these compounds. Polyphenolic compounds for example have been shown to possess antibacterial properties [36, 37]. Also, the presence of these phytoactive agents may perhaps account for the wide pharmacological effects attributed to *Aspilia Africana.*
The antibacterial sensitivity test, showed that all the extracts had antibacterial activity. However, ethanol extract of *A. africana* was more potent against the isolates than other extracts as shown in the work done by [38]. The highest mean diameter zone of inhibition for all the extracts was 15mm at 250mg/ml for *E. coli*. This conforms to the study done by [36]; although in our study the inhibition was seen *E. coli* while their study revealed against Staphylococcus species. The least inhibition observed was 0.7mm at 31.25mg/ml for *Pseudomonas aeruginosa.* The minimum inhibitory concentration (MIC) of all the extracts were obtained at 31.25mg/ml. This shows all the extracts contains antibacterial properties which enables them exert inhibitory effect on the bacterial isolates.

4.0 Conclusion

Aspilia africana holds a promising potential source for the development of new drug for treating bacterial infection. The antibacterial activity of the plant could be increased if the components are purified, so further research should be carried out to know the effect of different solvent in extracting the bioactive constituent of *A. africana*. Researchers should collaborate with traditional herbal practitioners to discover more about the usefulness and benefits of *Aspilia africana*.

References

[1] Hugo, W.B. and Russell, A. D (2003). *Pharmaceutical Microbiology*. 6th Edition: Blackwell Science Publishers
[2] Chong, K. T. and Pagano, P. J. (1997). In vitro combination of PNV-140690, a Human Immunodeficiency Virus type 1 Protease Inhibitor with Ritonavir against Ritonavirsensitive and Resistant Clinical Isolates. *Antimicrobial. Agents Chemotherapy*. **41** (2): 2367-2377.
[3] Aiyegoro, O. A. and Okoh, A. I. (2009). Use of Bioactive Plant Products in Combination with Standard Antibiotics: Implications in Antimicrobial Chemotherapy. *Journal of Medicinal Plant Research*. **3**(13):1147-1152.
[4] World Health Organization (WHO) (2002). Antimicrobial Resistance. Fact sheet No.194.
[5] Stermitz, F. R., Tawara-Matsuda, J., Lorenz, P., Mueller, P., Zenewicz, L. and K. Lewis, (2000). 5'-Methoxyhydnocarpin-D and Pheophorbide A: Berberis species components that potentiate berberine growth inhibition of resistant *Staphylococcus aureus*. *Journal of National* Product. **63**:1146-1149.
[6] Ahmad, I. and Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal Plants ESBL-producing multidrug-resistant enteric bacteria. *Microbiology Research*. **162**:264-275.
[7] Vadhana, P., Singh, B. R., Bharadwaj, M. and Singh, S. V. (2015). Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. *Pharmaceutica Analytica Acta*. **6**:434.
[8] Woodford, H. J. and George, J. (2011). "Diagnosis and management of urinary infections in older people". *Clinical Medicine*. London. **11**(1):80-83. doi:10.7861/clinmedicine.11-1-80. PMID 21404794.
[9] Centre for Disease Control, (2015). "Urinary Tract Infection". CDC. *Archived* from *the original on 22 on 22 February 2016. Retrieved 9 February 2016*
[10] Salvatore, S., Cattoni, E., Siesto, G., Serati, M., Sorice, P. and Torella, M. (2011)."Urinary tract infections in women." *European journal of obstetrics, gynecology, and reproductive biology*. **156** (2): 131–6. doi:10.1016/j.ejogrb.2011.01.028. PMID 21349630.
[11] Flores-Mireles, A. L; Walker, J. N; Caparon, M. and Hultgren, S. J. (2015). "Urinary tract infections: epidemiology, mechanisms of infection and treatment options".Nature Reviews. Microbiology. **13** (5): 269–84. *doi:10.1038/nrmicro3432*. *PMC 4457377*. *PMID 25853778*
[12] Elsevier Health Sciences (2015). Introduction to Medical-Surgical Nursing. p. 909 ISBN 9781455776412.
[13] Nicolle, L. E. (2008). "Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis". Urologic Clininc of North America. **35** (1): 1–12, *doi:10.1016/j.ucl.2007.09.004*. *PMID 18061019*.
[14] Jarvis, W. R. (2007). Bennett & Brachman's hospital infections.5th edition. Philadelphia: *Wolters Kluwer Health/Lippincott Williams & Wilkins*. p. 474. ISBN 9780781763837.

[15] Jarvis, T. R., Chan, L. and Gottlieb, T. (2014).”Assessment and management of lower urinary tract infection in adults”. *Australian Prescriber*. **37**(1):7-9.
[16] Asadi Karam, M. R., Habibi, M. and Bouzari, S. (2019). Pathogencity, antibiotic resistance and development of effective vaccine against Uropathogenic *Escherichia coli*. *Molecular Immunology.* **108**:56-67
[17] Kornfalt Isberg, H., melander, E., Hedin, k., Molstad. S. and Beckman, A. (2019). Uncomplicated urinary tract infections in Swedish primary care; etiology, resistance and treatment. *BMC infect. Dis*. 19(1):155
[18] van Pinxteren, B., Knottnerus, B. J., Geerlings, S. E., Visser, H. S., Klinkhamer, S. and van der Weele, G. M., Veruijn, M. M., Opstelten, W., Burges J. S. andVan Asselt, K. M. (2013). NHG-Standaard Urineweginfecties (derde herziening). Dutch College of General Practitioner’s guideline urinary tract infections (Third revision). *Huisarts wet*. 56(6):270-280.
[19] Colgan, R., Williams, M., and Johnson, J. R. (2011). "Diagnosis and treatment of acute pyelonephritis in women.". *American family physician.* **84**(5): 519–26. PMID 21888302.
[20] Sabih, A. and Leslie, W. (2020). Complicated tract infections. In: *StatPearls. Treasure Island (FL)*: StatPearls Publishing
[21] Hong, R., Xiao, L., Zhao-Hui, N., Jian-Ying, N., Bin, C., Jie, X., Hong, C., Xiao-Wen, T., Ai-MIM, R., Ying, H., Chang-Ying, X., Yinh-Hong, L., Yan-Feng, L., Jun, C., Rong, Z., Xu-Dong, X., Xiao-Hui, Q. and Nan, Chen. (2017). Treatment of complicated urinary tract and acute pyelonephritis by short-course intravenous levofloxacin (750mg/day) or conventional intravenous/oral levofloxacin (500mg/day): prospective, open-label, randomized, controlled, multicenter,non-inferiority clinical trial. *International Urology and Nephrology*. **49**(3):499-507.
[22] Jensen, W. B. (2007). "The Origin of the Soxhlet Extractor". *Journal of Chemical Education*. **84** (12):1913–1914. *Bibcode*:*2007JChEd..84.1913J*. *doi*:*10.1021/ed084p1913*.
[23] Sofowora, H. (1993). Screening Plants for Bioactive Agents In: Medicinal Plants and Traditional Medicine in Africa, *Spectrum Books* Ltd., Sunshine House, Ibadan. Nigeria.
[24] Harbone, N. V. (1994). Phytochemical methods. A guide to modern techniques of plant analysis 2nd Edition, *London Chapman and Hall*, London. pp 271.
[25] Trease, G. E. and Evans, W. C. (2001). *A Textbook of Pharmacognosy*, 14th Edition. W.B. Saunders Company Ltd. London; Pp 193-201, 232-233, 249-252.
[26] Velioglu, Y. S, Mazza, G., Gao, L. and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry.* **46**: 4113–4117.
[27] Makkar, H. P., Bluemmel, M., Borowy, N. K., and Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of Science and Food Agriculture*. **61:**161–165.
[28] Kumaran, A. and Karunakaran, J. (2006). In vitro anti-oxidant activities of methanol extracts of five Phyllanthus species from India. *LWT-Food Science. Technology.* **40:**344–352.
[29] Giusti, M. M. and Wrolstad, R. E. (2001). Characterisation and measurement of anthocyanins by UV-visible spectroscopy. In: R. E. Wrolstad (Ed.). *Current Protocols in Food Analytical Chemistry.* New York: John Wiley and Sons. pp 45-65.
[30] Wolfe, K., Xianzhong, W. and Rui, H. L. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*. **51**: 609−614 609.
[31] Perex, C. C., Paul, M. L. and Bazerque, P. (1990). Antibiotic Assay by Agar well diffusion Method. *Experimental Medicine and Biology*. **15**: 113-15.
[32] Alade, P. I. and Irobi,N. O. (1993). Antibacterial Activities of Crude Extracts of *Acalypha wilsiana*. *Journal of Ethnopharmacology*. **39**: 171-174.
[33] Nweze, E. I and Onyishi, M. C. (2010). In vitro Antimicrobial Activity of ethanolic and methanolic extracts of Xylopia aethiopica and its combination with disc antibiotics against Clinical Isolates of Bacteria and Fungi. *Journal of Rural and Tropical Public Health.* **9**:1-6.
[34] Clinical and Laboratory Standards Institute, (2015). Performance Standards for Antimicrobial Susceptibility Testing; *Twenty-Fifth Informational Supplement CLSI document* M100-S25. **35**:3
[35] Ebana, R. U. B., Edet, U. O., Ekanemesang, U. M., Etok, C. A., Ikon, G. M. and Noble, M. K. (2016). Phytochemical Screening and Antimicrobial Activity of Three Medicinal Plants against Urinary Tract Infection Pathogens. *Asian Journal of Medicine and Health*. **1**(2): 1-7.
[36] Li, F., WenQing, L. and XiaoMin, Z. (2016). Phenolic Compounds and In Vitro Antibacterial and Antioxidant Activities of Three Tropic Fruits: Persimmon, Guava, and Sweetsop *Biomedical Research International*. **3**:1-9. doi: 10.1155/2016/4287461
[37] Mandal, S. M., Dias, R. O. and Franco, O. L. (2017). Phenolic compounds in antimicrobial therapy. *Journal of Medicinal Food*. **20**(10):1031-1038. doi: 10.1089/jmf.2017.0017
[38] Anibijuwon, I. I., Duyilemi, O. P. and Onifade A. K. (2010). Antimicrobial activity of leaf of *Aspilia africana* on some pathogenic organisms of clinical origin. *Nigerian Journal of Microbiology*. **24**(1): 2048-2055.