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ANTIBIOTIC CHARACTERIZATION AND STUDY OF THE SENSITIVITY OF SOME BACTERIAL STRAINS OF URINARY ORIGIN TO FLUEGGEA VIROSA (ROXB, EX WILLD.) ROYLE LEAF EXTRACTS

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ABSTRACT

The increasing resistance of bacteria to antibiotics in the fight against urinary infections in recent decades constitutes a major public health concern. The objective of this study is to evaluate the antibacterial activity of *Flueggea virosa* leaves on multi-resistant urinary strains (*Escherichia coli*, *Klebsiella spp*, *Proteus mirabilis* and *Staphylococcus spp*). First of all, antibiotic profile of the bacterial strains was established according to the recommendations of the antibiogram committee of the French Society of Microbiology (CA-SFM). Then, antibacterial activity of aqueous and 70% hydro-ethanolic extracts of leaves of the plant was determined by punch well method in agar and dilution in liquid medium. The antibiotic profile revealed that all strains studied were multi-resistant with a minimum of 5 antibiotics. On the other hand, all the strains were very sensitive to *F. vorosa* extracts with diameters of the inhibition zones varying from 11.00 ± 1 mm to 22.00 ± 1 mm for the aqueous extract and 10. 66 ± 1.52 mm to 23.00 ± 0 mm for the hydro-ethanolic extract. Furthermore, the two extracts were active on all strains with MICs between 3.125 mg/ml and 12.5 mg/mL and MBCs oscillating between 3.125 mg/mL and 25 mg/mL. The MBC/MIC report indicates that both extracts had bactericidal power. These data would therefore justify the traditional use of this plant in the treatment of urinary and gynecological conditions in the North of Côte d'Ivoire.

KEYWORDS: *Flueggea virosa*; Multi-resistance urinary strains; Antibacterial activity; bactericidal.

INTRODUCTION

Urinary infections correspond to colonization of the urinary tract by one or more pathogenic bacteria. This attack on the tissue of urinary tree is characterized by an inflammatory reaction with symptoms of varying intensity and nature depending on the responsible organism.[1] The *Escherichia coli* strain is the bacteria most involved in these infections with a rate of 75 to 85%, followed by *Klebsiella spp* and *Proteus mirabilis*, which are responsible for approximately 8 to 12% of cases.[2] Urinary tract infections are 15 times more common in women than in men due to the short length of their urethra. This reduced size of the urethra in women facilitates the migration of intestinal bacteria from the anus to the bladder to create damage. Either way, urinary infections untreated or poorly treated can spread to the kidneys, blood, brain, and reproductive system. This can lead to serious complications such as pyelonephritis, sepsis, meningitis or infertility.[3]

In order to effectively fight against this multitude of germs, modern medicine has implemented several therapeutic treatments which use a variety of antibiotics. Indeed, antibiotic therapy has revolutionized the treatment of bacterial infections, saving many lives. Unfortunately, the excessive and inappropriate use of antibiotics has led to an increase in the resistance of different microorganisms to antibiotics. This antibiotic resistance then becomes a public health problem, thus prompting the search for new antibacterial molecules to neutralize multi-resistant germs.[4] It is with this in mind that our study falls, the objective of which is to fight against urinary infections caused by multi-resistant strains using extracts of *Flueggea virosa* leaves. Indeed,

previous studies have revealed that this plant is already used in the traditional treatment of several pathologies, notably gynecological infections in northern Côte d'Ivoire.[5,6]

MATERIAL AND METHODS

Material

Plant material

The plant material consisted of the leaves of *Flueggea virosa* harvested in Korhogo (Northern Côte d'Ivoire) in the morning using a knife on July 2023. After harvest, the organs of the plant were authenticated using National Floristic Center of the Félix HOUPHOUËT-BOIGNY University of Abidjan (Côte d'Ivoire) where they are kept under the herbarium number UCJ006375. Then, leaves were dried away from the sun at room temperature for 30 days in a room at Peleforo GON COULIBALY University in Korhogo (Côte d'Ivoire). After drying, they were pulverized with an electric grinder (RETSCH, Type AS 200) to obtain a fine powder, used for vegetal extracts preparation.

Bacterial strains

The bacterial strains used in this study were provided by the microbiology laboratory of Regional Hospital Center of Korhogo. These are precisely three strains of *Escherichia coli* (8039, 8133 and 8312); *two strains* of *Klebsiella spp* (6577 and 6580); *two strains* of *Proteus mirabilis* (7713 and 7722) and two strains of *Staphylococcus spp* (9044 and 9109). After receipt of the different bacterial strains, their were storaged at -20°C before use.

Methods

Preparation of plant extracts

The tests focused on the aqueous and 70% hydro-ethanolic extracts. These two extracts were prepared according to the method described by Ouattara *et al.* (2012).[7] Indeed, 100 g of leaf powder were macerated in 1 L of distilled water or in 1 L of ethanol 70%. The whole is then homogenized in a Nasco brand blender (BL1008A-CB). The homogenate obtained is drained through white percale fabric and double filtered through hydrophilic cotton. The filtrate obtained was concentrated at 50 °C in an oven until solvent had completely evaporated in order to obtain dry aqueous and 70 % hydro-ethanolic extracts.

Preparation of bacterial inoculum

To prepare the inoculum of each bacterial strain, two young colonies aged 18 to 24 hours were homogenized in 10 mL of Mueller-Hinton broth then incubated for 3 hours at 37°C. After incubation, 0.1 mL of the broth was added to 10 mL of sterile distilled water to constitute the inoculum estimated at 5.10^6 bacteria/mL with a turbidity 0.5 Mac Farland.

Determination of strains antibiotic profile

The isolated strains underwent antibiogram test using diffusion method in agar medium according to the recommendations of Antibiogram Committee of the French Society of Microbiology (CA-SFM).[8] Strains were tested for sensitivity to the following antibiotics: erythromycin (15 µg); gentamycin (30µg); amoxicillin (30µg); tetracycline (30µg); ceftriaxone (30µg); colistin (30µg); ciprofloxacin (5µg); sulfamethaxazole/Trimethoprim (23.75 µg); metronidazole (16 mcg) and cefoxitin (30 mcg). For this, a drop of the already prepared bacterial inoculum was added to 9 mL of physiological water then homogenized using a vortex. The obtained homogenate was used to flood the Mueller Hinton agar in a Petri dish. After flooding, it was dried in an oven for 15 to 30 min at 37°C after aspiration of excess liquid using a sterile Pasteur pipette. Then, the disks impregnated with antibiotics were placed on surface of the agar by gently applying them with sterile forceps. Inhibition diameters were read after 18 to 24 hours of incubation at 37°C and their interpretation (Sensitive or resistant) was made following a reference table. Finally, the rate of resistance of the strains to antibiotics was calculated using the following formula:

$$\text{Antibiotic resistance rate} = \frac{\text{Total number of resistant strains x 100}}{\text{Total number of strains studied}}$$

Evaluation of the sensitivity of strains to *F.virosa* extracts

Sensitivity of the strains to the extracts was determined by agar punch well method in a Petri dish. For this, Mueller Hinton agar was first inoculated by flooding with the inoculum prepared beforehand. Then, it was dried in an oven for 15 to 30 min at 37°C after aspiration of excess liquid using a sterile Pasteur pipette. Wells of 6 mm in diameter and separated by at least 20 mm were made in the agar using a sterilized Pasteur pipette. Finally, each well was filled with 80 µL of aqueous extract or hydro-ethanolic extract at 100 mg/Ml.[9] A control well was prepared with 80 mL of a mixture of DMSO/sterile distilled water (v/v). At the same time, gentamycin (30 µg) was used as a standard positive control antibiotic. After 45 min of pre-diffusion, the whole was incubated in an oven at 37°C for 24 hours. The effect of each extract on strain studied was assessed by measuring the diameter of the growth inhibition zone around the well. Strain is sensitive to the extract if this diameter is less than 10 mm and otherwise, it is said to be resistant.[10] This test was carried out in triplicate for each extract.

Determination of antibacterial parameters

Preparation of the concentration range

A range of concentrations of each extract, from 100 to 0.78 mg/mL was prepared by double dilution method in test tubes.[11] Thus, 1000 mg of fine powder of the extracts were mixed with 10 mL of distilled water to constitute the initial concentration C_1 = 100 mg/mL. Then, 5 mL of this solution were added to 5 mL of distilled water to obtain the concentration C_2 = 50 mg/mL and we proceeded as follows to obtain the other

concentrations: C_3 = 25 mg/mL; C_4 = 12.5 mg/mL; C_5 = 6.25 mg/mL; C_6 = 3.125 mg/mL; C_7 = 1.56 mg/Ml and C_8= 0.78 mg/mL. The contents of the tubes thus prepared were sterilized at 121°C for 15 min in the autoclave.

Determination of the minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC), 1mL of each inoculum and 1mL of the concentration of each plant extract were introduced into a hemolysis tube and homogenized. This operation was carried out for each of the concentrations prepared. These tubes were then incubated at 37°C for 18 to 24 hours. After incubation, the MIC corresponding to the lowest concentration which induces an absence of bacteria grow visible to the naked eye, was determined by observing the tubes which contain the plant extracts.

Determination of minimum bactericidal concentration

To determine the minimum bactericidal concentration **(**MBC), two Petri dishes (A and B) were used, each containing Mueller-Hinton agar. The dish A was inoculated in parallel streaks of 5 cm, with 0.1 mL of contents of each of tubes having a concentration greater than or equal to the MIC using a sterile calibrated loop. At the same time, dilutions from the mother suspension (100) were made up to the dilution 10^{-4}. Then, these dilutions and the mother suspension were also inoculated by parallel streaks in dish B. The two boxes were incubated at 37°C for 18 to 24 hours. To determine the MBC, the different colonies in dish A were compared to those in the 10^{-4} dilution of Petri dish B. MBC corresponds to the concentration of the plant extract presenting a number of colonies in the dish A less than or equal to that of the 10^{-4} dilution of dish B. This MBC is the smallest concentration which allows at most 0.01% of the germs in the starting suspension to survive for 24 hours. Finally, the MBC/MIC ratio was calculated to determine the antibacterial power of each extract. An extract is judged to be bactericidal if this ratio is less than or equal to 4 and bacteriostatic if it is greater than 4.[12]

Statistical analysis

Statistical tests were carried out exclusively using SPSS software (statistics 26) and the data were entered using Word and Excel 2016 software. The significance of the differences observed between the different test groups is assessed by analysis of variances (ANOVA) of Turkey's multiple comparison test.

RESULTS

Antibiotic profile of bacterial strains

The antibiogram showed that all bacterial strains were multi-resistant with resistance to at least five (05) antibiotics. The most multi-resistant strains were the strains of *E. coli* and *P. mirabilis* with resistance to 9 antibiotics, while the least resistance was obtained with the strains of *Klebsiella* and *Staphylococcus spp* (6 antibiotics) **(Figure 1).** Indeed, β-lactam class antibiotics consisting of amoxicillin, ceftriaxone and cefoxitin were ineffective on all strains (100%). These germs were also more resistant to colistin, sulfamethaxazol/trimethoprim (90%) and tetracycline (80%) followed by Erythromycin, ciprofloxacin and metronidazole (70%). Compared to all these antibiotics, gentamicin was effective on the majority of strains with the lowest resistance rate (30%) **(Figure 2).**

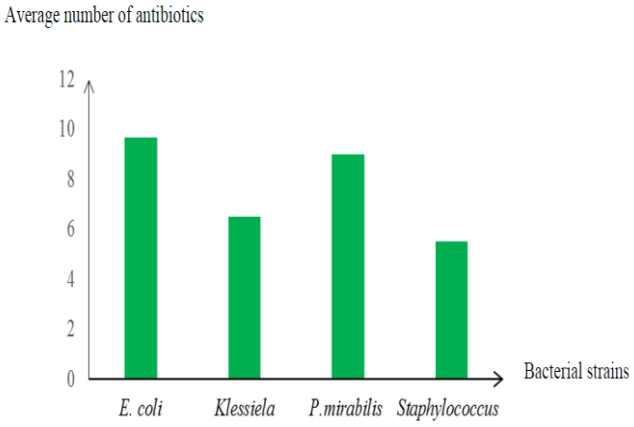

Figure 1: Number of ineffective antibiotics per bacterial strain.

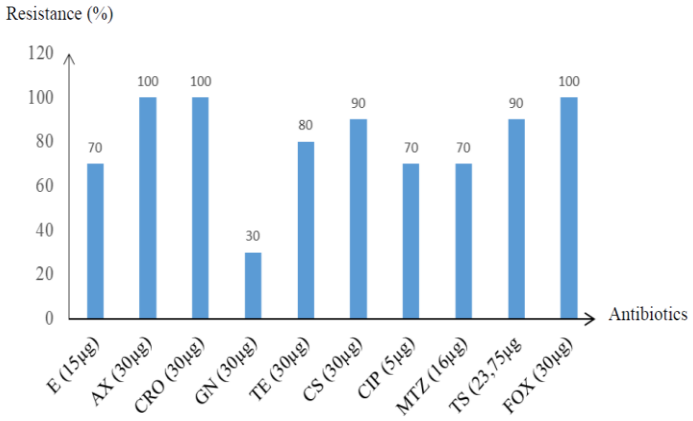

Figure 2: Resistance profile of urinary germs to different antibiotics. ***E: Erytromcyne; GN: Gentamicin; AX: Amoxicillin; TE: Tetracycline; CRO: Ceftriaxone; CS: Colistin; CIP: Ciprofloxacin; TS: Sulfamethaxazole/Trimethoprim; MTZ: Metronidazole; FOX: Cefoxitin.***

Antibacterial activity of *F.virosa* extracts

Sensitivity of strains to extracts

Table I presents diameters of inhibition of the strains by the plant extracts. Analysis of these results shows that aqueous and hydro-ethanolic extracts of *F.virosa* were active on all germs. Indeed, the inhibition diameters recorded with the aqueous extract varied from 11.00 ± 1.00 mm to 22.00 ± 1.00 mm while those of the hydro-ethanolic extract were between 10.66 ±1.52mm and 23.00±0.00mm. Statistical analysis revealed that there is no significant difference (p>0.05) between the effects of the two plant extracts. The activity of the aqueous and hydro-ethanolic extracts was more intense on *Staphylococcus spp* 9044 with respective inhibition zones of 23.00 ± 0.00 mm and 22.00 ± 1.00 mm and less intense on *P. mirabilis* with respective diameters of 10.66 ± 1.52mm and 11.00 ± 1.00.

Table I: Sensitivity of different strains to *F. virosa* extracts.

Bacteria	Code	Aqueous extract		Hydro-ethanolic extract	
		Inhibition diameter (mm)	Interpretation	Inhibition diameter (mm)	Interpretation
E.coli	8039	15.33 ± 0.58 *	Sensitive	16.00 ± 0.00 *	Sensitive
	8133	15.00 ± 0.00 *	Sensitive	15.00 ± 0.00 *	Sensitive
	8312	15.33 ± 1.53 *	Sensitive	15.00 ± 1.00 *	Sensitive
Klebsiella spp	6577	15.00 ± 1.00*	Sensitive	14.33 ± 0.58 *	Sensitive
	6580	19.33 ± 0.58*	Sensitive	17.33 ± 1.52 *	Sensitive
P.mirabilis	7713	15.33 ± 0.58*	Sensitive	16.66 ± 1.52 *	Sensitive
	7722	11.00 ± 1.00*	Sensitive	10.66 ± 1.52 *	Sensitive
Staphylococcus spp	9044	22.00 ± 1.00*	Sensitive	23.00 ± 0.00 *	Sensitive
	9109	18.66 ± 1.53*	Sensitive	19.00 ± 1.00 *	Sensitive

(*) = p> 0.05: no significant difference between the effect of the aqueous and hydro-ethanolic extract

Antibacterial parameters of extracts

The antibacterial parameters of the extracts are recorded in **Table II**. It appears that aqueous and hydro-ethanolic extracts of *F.virosa* inhibited bacterial growth after 18 to 24 hours of incubation at minimum inhibitory concentrations (MICs) between 3.125 mg/ mL and 12.5 mg/mL. For both extracts, the lowest MIC (3.12 mg/ mL) was observed with the strains of *E.coli* 8312, *Klebsiella spp* 6580 and *Staphylococcus spp* 9109 while the strains *E.coli* 8039, *E.coli* 8133, *Staphylococcus spp* 9044 recorded the highest value (12.5 mg/ mL).

Concerning the minimum bactericidal concentrations (MBCs) obtained with boxes A and B, the values were between 3.12 mg/mL and 25 mg/mL. Thus, with the aqueous extract, the lowest MBC (6.25 mg/mL) was obtained with *Staphylococcus spp* 9109 followed by the two strains of *Klebsiella* spp and *E.coli* 8312 (12.5 mg/mL). For the rest of the strains studied, the MBC was 25 mg/mL. The same observation was made with hydro-ethanolic extract with exception of *Staphylococcus spp* 9109 which recorded the lowest MBC (3.12 mg/ mL) followed by the two *Klebsiella* 6580 and *E.coli* 8312 (12 .5 mg/mL). For the rest of the strains studied, the MBC was 25 mg/mL. Furthermore, the two extracts showed bactericidal power on all the strains tested. Indeed, the MBC/MIC ratio varied between 1 to 4, therefore less than or equal to 4 for all the strains.

Table II: Antibacterial parameters of *F. virosa* extracts on multi-resistant bacteria.

Extracts	Bacteria	Codes	Paramètre antibactériens			
			MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	Antibacterial power
Aqueous	*E.coli*	8039	12.5	25	2	Bactericidal
		8133	12.5	25	2	Bactericidal
		8312	3.12	12.5	2	Bactericidal
	Klebsiela spp	6577	6.25	12.5	2	Bactericidal
		6580	3.12	12.5	4	Bactericidal
	P.mirabilis	7713	6.25	25	4	Bactericidal
		7722	6.25	25	4	Bactericidal
	Staphy Spp	9044	12.5	25	2	Bactericidal
		9109	3.12	6.25	2	Bactericidal
Hydro-ethanolic	*E.coli*	8039	12.5	25	2	Bactericidal
		8133	12.5	25	2	Bactericidal
		8312	3.12	12.5	4	Bactericidal
	Klessiela	6577	6.25	25	4	Bactericidal
		6580	3.12	12.5	4	Bactericidal
	P.mirabilis	7713	6.25	25	4	Bactericidal
		7722	6.25	25	4	Bactericidal
	Staphy Spp	9044	12.5	25	4	Bactericidal
		9109	3.12	3.12	1	Bactericidal

DISCUSSION

The bacteria studied were all multi-resistant with a minimum resistance level of five antibiotics. None of the bacteria were sensitive to β-lactams, including amoxicillin, ceftriaxone and cefoxitin. This resistance to β-lactams and other antibiotics could be due to excessive and inappropriate use of these antibiotics. These results are similar to those obtained in Mali.[13] Indeed, these researchers have highlighted the multi-resistance of the bacteria responsible for urinary infections. These bacterial strains showed 93.31% resistance to amoxicillin, 76.09% to third generation cephalosporins and 65.22% to ciprofloxacin. Antibiotics kill bacteria, but some survive and pass their resistance on to their descendants. Thus, antibiotic resistance is spreading in the population. Finally, this antibiotic resistance complicates the management of infectious diseases, because the number of multi-resistant bacteria has continued to increase in recent years.[14] Compared to usual antibiotics, hydro-ethanolic and aqueous extracts of *F.virosa* were more active on all strains. Indeed, all the strains studied were highly sensitive to the two extracts (aqueous and hydro-ethanolic) which inhibited their growth at relatively low MIC and MBC with bactericidal power. The antibacterial activity exerted by *F.virosa* leaf extracts could be explained by the presence of a diversity of secondary metabolites in aerial parts of this plant. Indeed, *F.virosa* leaves have been shown to contain total polyphenols, flavonoids, gallic tannins, catechin tannins and saponins.[15,16] These phytomolecules have already been recognized for their antibacterial activity against multi-resistant strains. These results corroborate those of Kouadio *et al.* (2015) who showed the bactericidal activities of aqueous and hydro-alcoholic extracts of *Mallotus oppositifolius* leaves on multi-resistant bacteria involved in various infections, including urinary infections.[17] Indeed, the phytochemical characterization of extracts from the leaves of this plant also revealed the presence of a large number of phenolic compounds with well-known antibacterial properties.[18,19] Indeed, tannins and flavonoids have the capacity to inhibit the growth of several bacteria.[20] These results are to be encouraged because *F.virosa* leaves could be used to develop new antimicrobials to effectively fight against multi-resistant germs.

CONCLUSION

The aim of the study was to antibiotypically characterize bacterial strains of urinary origin and to evaluate the antibacterial effects of aqueous and 70% hydro-ethanolic extracts of *Flueggea virosa* leaves on these strains. At the end of this work, it emerged that all the strains were multi-resistant to antibiotics. Furthermore, the strains were sensitive to the two plant extracts with a bactericidal effect. These results can serve as a solid basis for the implementation of traditionally improved drugs to effectively combat urinary tract infections.

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REFERENCES

1. Alrasheedy M, Abousada HJ, Abdulhaq MM, Alsayed RA, Alghamdi KA, Alghamdi FD, Muaibid AF, Ajjaj RG and Almohammadi SS. Prevalence of urinary tract infections among children in the Kingdom of Saudi Arabia. Artch Ital Urol Androl, 2021; 93(2): 206-210.

2. Barber AE, Norton JP, Spivak AM and Mulvey MA. Urinary tract infections: current and emerging management strategies. Clinical Infectious Diseases, 2013; 57(5): 719-724.
3. Bruyere F, Bey E, Cariou G, Cattoir V, Saint F, Sotto A and Vallée M. Urinary infections in adults: comparison of French and European recommendations. By the Infectiology Committee of the French Urology Association. Science Direct, 2020; 30: 472-481.
4. WHO. WHO/ECDC report, antimicrobial resistance threatens patient safety in the European Region, accessed 14.01.2024, at: https://www.who.int/europe/en/news/item/who-ecdc-report--antimicrobial-resistance-threatens-patient-safety-in-european-region.
5. Koman SR, Kpan WB, Yao K and Ouattara D. Plants used in the traditional treatment of female infertility in the department of Dabakala (Ivory Coast). J Anim Plant Sci, 2019; 42(1): 7086-7099.
6. Kpabi I, Agban A, Hoekou Y, Pissang P, Tchacondo T and Batawila K. Ethnobotanical study of plants with antiparasitic activities used in traditional medicine in the prefecture of Doufelgou in northern Togo. J Appl Biosci, 2020; 148: 15176-15189.
7. Ouattara K, Doumbia I, Coulibaly FA and Coulibaly A. Influence of Thonningia sanguinea (THOS) on the productivity of a laying hen farmInt. J. Biol. Chem. Sci, 2012; 6(5): 1960-1969.
8. CAS-FM (Antibiogram Committee of the French Microbiological Society). Recommendation 2016, available on the website www. sfm-microbiology.org/Userfile.
9. Ganfon H, Houvohessou JP, Assanhou AG, Bankole HS and Gbenou J. Antibacterial activity of the ethanolic extract and fractions of Anogeissus leiocarpa (DC) Guill. And Perr. (Combretaceae). Int J Biol Chem Sci, 2019; (2): 643-651.
10. Tsirinirindravo LH and Andrianarisoa B. Antibacterial activities of Dalechampia clematidifolia (Euphorbiaceae) leaf extract. Int J Biol Chem Sci, 2009; 3(5): 1198-1202.
11. Ouattara K, Doumbia I, Coulibaly AF, Siaka S and Coulibaly A. *In-vitro* antibacterial activity of *Thonningia sanguinea* [Balanophoraceae (Vahl)] flowers extracts. J. Microbiol. Biotech. Res, 2013; 3(2): 83-87.
12. Biyiti L, Meko'o D, Tamzc V and Amvam Zollo P. Recherche de l'activité antibactérienne de quatre plantes médicinales camerounaises. Pharm Méd Trad Afr, 2004; 13: 11- 20.
13. Diarra L, Diarra S, Sangaré A, Diepkile A, Sanogo A, Marico M, Doumbia S, Bagayoko M, Dembélé D, Doumbia T, Dissa M, Ouologuem I and Coulibaly S. Epidemiological and Bacteriological Profile of Urinary Tract Infections at the Medical Biology Laboratory of Sikasso Hospital. PriMera Scientific Medicine and Public Health, 2023; 23(12): 65-68.
14. Benhiba I, Bouzekraoui T and Zahidi J. Epidemiology and antibiotic resistance of enterobacterial urinary infections in adults in the Marrakech University Hospital and therapeutic implication. Uro Andro, 2015; 1(4).
15. Traoré K, Haidara M, Denou A, Kanadjigui F, Sogoba MN, Diarra B, Maiga S and Sanogo V. *(*Phytochemical Screening and Biological Activities of Four Plants Used in Mali in the Management of Malaria in Children. Eur Sci J, 2019; 15: 1857- 7881.
16. Soro T, Kamagaté T, Touré A, Méité S, Kablan ALC and Coulibaly A. Phytochemical screening and effects on spermatogenesis of extracts from leaves of *Flueggea virosa* (Roxb, ex Willd.) Royle and *Heliotropium indicum* L., two plants used against infertility in North of Ivory Cost. J Phytopharmacol, 2024; 13(1): 37-42.
17. Kouadio NJ, Guessennd NK, Kone MW, Moussa B, Koffi YM, Guede KB and Dosso M. Evaluation of the activity of the leaves of Mallotus oppositifolius (Geisel.) Müll.-Arg (Euphorbiaceae) on multi-resistant bacteria and phytochemical screening. Int J Biol Chem Sci, 2015; 9(3): 1252-1262.
18. Morel S.Phytochemical study and biological evaluation of Derris ferruginea Benth. (Fabaceae). Doctoral thesis, University of Angers, France, 2011; 263.
19. Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol, 2011; 23: 1-8.
20. Sepúlveda L, Ascacio A, Rodríguez-Herrera R, Aguilera-Carbó A and Aguilar CN. Ellagic acid: Biological properties and biotechnological development for production processes. Afr J Biotechnol, 2011; 10(22): 4518-4523.