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Polyphenolic and Flavonoids Contents and Antibacterial Activity of Hydro-Ethanolic and Aqueous Extract of Fresh Leaves of Gardenia Aqualla Staph and Hutch (Rubiaceae)

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Abstract: Erectile dysfunction has become for two decades, a real public health problem. Its prevalence is estimated at 322 million in 2025. Its management is based on the administration of phosphodiesterase inhibitors alone or in combination. Infections have been rarely mentioned although *E coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* or *Serratia marcescens* are recognized as factors of erectile dysfunction. The purpose of this study was to evaluate the phytochemical profile of the aqueous (AE) and hydroethanolic (HE) extracts of the fresh leaves of *Gardenia aqualla* and to determine their antibacterial potential on bacteria involved in male accessory gland infections. The aim of this study was to evaluate phytochemical profile of aqueous (AE) and hydroethanolic (HE) extracts of fresh leaves of *Gardenia aqualla* and to determine their antibacterial potential on bacteria involved in male accessory gland infections. Phytochemical profile highlighted the presence of alkaloids, polyphenols with concentrations of 434.5 ± 14.5 mgEq Tannic acid/g of dried extract greater than 249.5±18.3 mgEq Tannic acid/g of dried extract found in AE including flavonoids whose concentrations is 184.9 ± 6.3 µgEqRutin/g of dry extract in HE against 183.0±9.1 µgEqRutin/g of dried extract in AE. Antibacterial tests showed equivalence of activity on *E coli* and S aureus strains with MICs of 100 mg/mL and 50mg/mL respectively and only HE showed activity on *Klebsiella pneumoniae* and *Serratia marcescens* which had the lowest MIC 6.25 mg/mL.

Keywords: Fresh Leaves Extract, *Gardenia Aqualla*, Polyphénols, Flavonoïds, Male Accessory Glands Infection

1. Introduction

Disorders of sexuality, commonly called "sexual impotence", is a condition characterized by repeated inability to initiate or maintain a rigid erection during sexual intercourse [1] for at least three months [2]. Its worldwide prevalence ranges from 15% to 52% [3] depending on socio-economic status, toxic, anatomical or infectious conditions [4, 5]. The global prevalence of erection disorder (ED) is a growing issue. Estimated to affect 150 million individuals in 1994, the global prevalence increased to 322 million in 2025 [6, 7] corresponding to an incidence of 169% [6]. In Africa it is estimated at about 11.5 million in areas such

as South Africa where 69.4% of people over 18 years old are concerned [5]. In Tanzania a study conducted on 381 participants in 2020 revealed that about 29% of 40 years old male were affected by ED [8]. Main aetiologies of ED have physical and psychological origin [9, 10]. The management, although daily or punctual, is mainly based on phosphodiesterase-5 inhibitors used alone or in association or surgery [11], Very rarely antibacterial agents are mentioned in management, although bacterial infections are known to associate with ED development. [4].

Infections of the male accessory glands (prostate, seminal vesicle and epididymis) [4] are known to induce sexual disorders by testicular atrophy or by obstruction of the male genital tract [12] at the unset of prostatitis, prostate-vesiculitis and prostate-vesiculo-epididymitis [4, 13]. The germs involved are sexually transmitted and their pathogenicity results from their ability to resist conventional antimicrobial agents [14]. In fact, antibacterial resistance is one of the biggest health challenges that threaten global health, food security and overall development [15] with increased medical charges morbidity and mortality rates. New therapeutic targets are being explored alongside with traditional pharmacopoeia as an important source for research. Plants like *Pausinistalia yohimbe, Tribulus terrestris, Panax ginseng* have long been used traditionally for their activities and introduced into the therapeutic arsenal in in force in many countries for ED [16]. whose. On the contrary, some others like S*olanum* spp, *Gardenia aqualla Staph and Hutch* (Rubiaceae) are traditionally indicated in ED and in infectious diseases control.

Gardenia aqualla belongs to the large family of Rubiaceae. It is used by traditional practitioners for the caretaking of leprosy, infections of the oropharyngeal and auditory sphere [17] and gastrointestinal disorders [18] in Cameroon. In order to contribute to the valorisation of this plant in connection with the search for new antibacterial agents; The present investigation was undertaken to determine the chemical composition and to assess the antibacterial potential of Gardenia aqualla on Escherichia coli, *Klebsiella pneumoniae*, Enterobacter responsible for urinary tract infections often associated with prostate disorders (abscess, chronic bacterial prostatitis), *Staphylococcus aureus* implicated in epididymitis or prostatitis, *Serratia marcescens* (acute epididymitis, prostatitis) [13, 19, 20] and other disorders of the male accessory glands [4, 21]. These bacteria are also reported to be resistant to many conventional antibacterial drugs [22]. More specifically, parameters investigated will be include minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and MBC/MIC ratio. In the short run, findings from the present survey will orient traditional practitioners in their daily activities. In the intermediate and long runs, they will help advocate development of larger scale production of available and affordable alternatives to conventional antibacterial agents subsequent to related toxicity investigations.

2. Material

2.1. Plant material Culture Media

The fresh leaves of Gardenia aqualla were collected in the Bangoua, West Cameroon in February 2021 and identified in the National Herbarium of Cameroon under reference 35933 HNC. Culture media used included Mueller Hinton Broth (MHB) (for MIC) and Mueller Hinton agar (for MBC) agar.

2.2. Subjected Bacteria

Bacterial strains subjected in the present investigation were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC25923) *Klebsiella pneumoniae* (ATCC 13883) and *Serratia marcescens* (ATCC14756). These bacteria are known aetiologies of infections in reproductive accessory organs. All isolates were provided by the Microbiology Laboratory at the Université des Montagnes Teaching Hospital. These strains were chosen according to their involvement in infections of male accessory glands related to the development of ED [23].

3. Methods

3.1. Extraction from Fresh Leaves

Thoroughly washed with distilled water, fresh leaves were used in this part. For that purpose, leaves were cut into small pieces and used for extraction. Three hundred grams were macerated in the appropriate solvent (Water-ethanol mixture (30/ 70) or distilled water). The solvent was renewed every 24 hours during three days. Macerates were filtered through Whatman paper No. 3. Each resulting filtrate was concentrated under reduced pressure with a BÜCHI R-201 rotary evaporator at 38°C and subsequently dried in an oven at 40°C. The yield was the assessed. For 18.34 g (yield of 6.11%) of dark green, hygroscopic powder representing the hydro-ethanolic extract (HE) or 12.97 g (yield of 4.33%) of dark brown colour corresponding to aqueous extract (AE).

3.2. Phytochemical Screening of Aqueous and Hydro-Ethanolic Extracts of Gardenia Aqualla

Test for secondary metabolites in the extracts were performed according to Bassene and Bruneton [24]. Namely they were flavonoids (Shinoda test), tannins (Stiasny reaction followed by that of ferric chloride), alkaloids (Dragendorff and Mayer reagent), sterols and terpenes (Liebermann-Buchard reaction), saponosides (foam index) and quinones.

3.2.1. Test for Polyphenol: Perchloride Test

Into a test tube containing 1 mL of ethanol, 5 mg of extract was added and thoroughly mixed. Into this mixture, 3 drops of 10% iron perchloride were added. Development of an intense purplish-blue colour indicated the presence of polyphenols.

3.2.2. Test for Flavonoids: Shinoda's Test

Into a test tube containing 1 mL of ethanol, 5 mg of the extracts were added and thoroughly stirred. To the resulting

preparation 1mL of water, 0.5 mL of iso-amylic alcohol, 0.05 g of magnesium shavings and 3 drops of concentrated H_2SO_4 were added thereafter. Development of a pink red colour reflected the presence of flavonoids.

3.2.3. Test for Alkaloids

To be conducted, 5 mg of each extract was dissolved in 5 mL of distilled water. Thereafter, 1 mL of 2% hydrochloric acid was added. The acidified solution was divided into two test tubes for Mayer's reagent and Dragendorff reagent tests.

Mayer's reagent test

Into the first tube, 5 drops of Mayer's reagent were added. A white squinty precipitate indicated the presence of alkaloid.

Dragendorff reagent test

Into the second above mixture, 5 drops of Dragendorff's reagent were added. A brownish precipitate clearly separated from the solution with the reagent indicated a positive test for the presence of alkaloids.

3.2.4. Test for Saponins

For each extract 5 mg was dissolved in 10 mL of distilled water within a test tube. The mixture was stirred vertically for about 15 seconds, then let to stand. Development of a one-centimeter persistent foam reflected a positive test for the presence of saponins in the extracts.

3.2.5. Test for Steroids and Terpenoids

Into 1mL of each extract prepared in distilled water, 1 mL of methanol was added. To the resulting mixture, 0.2 mL of each of the following reagents was sequentially dispensed: chloroform, glacial acetic anhydride and concentrated H_2SO_4. Development of an intense purple color indicated positive tests for the presence of steroid in the extract. When trichloracetic acid was thereafter added, a yellow color that turned into red indicated positive tests for terpenoids in the extract.

3.2.6. Test for Tannins

In 1 mL of the reconstituted extract, 1 mL of methanol was added. To the resulting preparation, five drops of the Stiasny reagent were dispensed, and the mixture centrifuged. A precipitate formed indicated the presence of condensed tannins. To the supernatant obtained from above centrifugation, five drops of a 1% ferric chloride solution were sequentially added. Development of a blue-blackish staining color indicated the presence of hydrolysable tannins.

3.2.7. Test for Anthocyanins

In a test tube containing 1 mL of distilled water, 5 mg of extract were dissolved. To the mixture, 1 mL of concentrated hydrochloric acid and 1 mL of 25% ammonia were sequentially dispensed. Positive tests for the presence of anthocyanins in the extracts were indicated by the development of a purplish-blue color.

3.2.8. Test for Anthraquinones

About 5 mg of extract solution were diluted in 4 mL of a mixture of chloroform and petroleum ether (v/v). the preparation was thereafter, the homogenized and filtered. Into 1 mL of the filtrate, 1 mL of a 10% NaOH solution was added. Development of a red color indicated positive tests for anthraquinones.

3.3. Quantification of total Phenolic and Flavonoid

The quantification technique was primarily validated according to the ICH concept by assessing the linearity, accuracy, precision, Limit of Detection (LOD) and Limit of quantification (LOQ) [25]. Linearity was established through a calibration curve drawn by linear regression of absorbance of dilutions from a standard solution measured 3 times. Linearity was established if the R^2 was greater than 0.996. Accuracy was assessed by determination of the recovery rate (%) while precision was performed by measurement of 2 concentrations of analytes 6 times per day during 2 different days and determining the variation coefficient. Comparison tests of ordinate with origin and existence of significant slope were conducted with t-student test [26, 27].

3.3.1. Total Phenolic Content

Total phenolic contents were assessed according to Singleton and Ross (1965) with slight modifications. Briefly, 100 µL of the extract were brought into contact with 500 µL of diluted (1/10) Folin-Ciocalteu reagent. After 2 minutes of incubation, 2 mL of a 20% sodium carbonate was added. The mixture was shaken thoroughly and let to stand for 30 min at room temperature. Absorbance was thereafter, read at 730 nm. The polyphenol was quantified based a calibration curve developed under the same operating conditions with tannic acid as standard, prepared at concentrations ranging from 25 to 125 µg/mL [28].

3.3.2. Quantification of Total Flavonoids

Quantification was performed according to Zhishen et al. (1999) and Kim et al. (2003) with slight modifications [29, 30]. In each case and sequentially, 200 µL of extract, standard or distilled water were mixed with 800 µL of methanol. Then, 50 µL of 10% $AlCl_3$ and 50 µL of saturated solution of sodium acetate were added subsequently. The resulting preparation was thoroughly mixed with a vortex mixer and incubated. After 6 minutes of incubation, 1400 µL of distilled water were added and the whole homogenized with a vortex stirrer. Absorbance was read immediately at 510 nm. The flavonoid contents were then determined with respect to the calibration curve of the dilutions of a methanolic solution of rutin (as standard) for which the concentrations were found between 20 and 100 µg/mL.

3.4. Tests for the Antibacterial Potential

Testing antibacterial activity prior to MIC and MBC was conducted according to the routine antibiogram principle. To 6 mm diameter sterile discs firmly adjusted on fresh bacterial culture streaked on Mueller Hinton, 50 µL of the extract (200 mg/mL) were dispensed. The set was then incubated at 37°C overnight. Upon completion of incubation, the presence of an inhibition zone around the paper disk testified the antibacterial

activity of the extract [31–33].

3.4.1. Preparation of Fresh Bacterial Inoculum

In aseptic environment, bacteria were sub-cultured on nutrient agar, then incubated at 37°C for 24 hours. From the fresh bacterial subpopulation obtained upon completion of incubation, a few colonies were used to prepare a suspension with an opacity similar to 0.5 on the McFarland turbidity ($1.5x10^5$-10^8 CFU) in sterile physiological saline [34].

3.4.2. Test for the Minimal Inhibitory Concentration (MIC)

Determination of the MIC was based on the susceptibility of subjected bacteria to extracts, the lowest concentration which inhibits visible bacterial growth in convenient growth environments. In the present study bacterial were grown in Mueller Hinton broth and incubation at 37°C for 18 to 24 hours [31, 32]. A serial twofold-dilution (1/2) was conducted from 200 mg/mL (the original) through 3.125 mg/mL in glass test tubes. From the bacterial inoculum previously made, 15 µL were dispensed in each tube subsequently incubated for 24 hours at 37°C. In each case, the MIC was identified as the first test tube of the range (the least concentrated) in which no turbidity was recorded (absence of turbidity) upon completion of incubation. Each test was conducted three times.

3.4.3. Test for the Minimal Bactericidal Concentration (MBC)

The MBC represents the lowest antibacterial agent concentration which kills more that than 99.9% of the initial population upon completion of incubation conducted in convenient growth conditions. In the present study, it was conducted on Mueller Hinton agar at 37°C for 18 to 24 hours. Assessment of MBC was based on subculture from the MIC conducted on Mueller Hinton agar. Preparations in test tubes for which no turbidity was observed were resuspended by stirring. From the resuspended preparation, 10 µL were steaked on Muller Hinton agar in Petri dishes. The set was thereafter, incubated at 37°C overnight. Upon completion of incubation, MBC was identified as the lowest concentration the MIC test tubes from which no growth was recoded on Muller Hinton. Each test was repeated three times. The bacteriostatic and bactericidal potential were evaluated by computing MBC/MIC ratio. When MBC/MIC ratio was higher than 4, the extract was qualified as bacteriostatic. When it was lower or equal to 4, it was said to be bactericidal and absolute bactericidal when it was equal to 1 [32].

4. Results

4.1. Extraction

The table 1 provides pieces of information on the form the two variants of extraction (aqueous (AE) and the other hydro-ethanolic 30 – 70 (HE)) performed with the leaves of *Gardenia aqualla Staph & Hutch* and the related yields.

Table 1. *Extraction yield (Water and Mixture Water-Ethanol extracts).*

Extraction	Dried extract weight (g)	Fresh leaves weight (g)	Yield (%)
AE	12.97	300	4.33
HE	18.34	300	6.11

It can be observed that the extraction yield is relatively higher with the hydro-ethanolic extract.

4.2. Phytochemical Screening

Investigating through secondary metabolites, related pieces of information on the semi-quantification in both extracts were summarized and presented as shown in table 2.

Table 2. *Phytochemical screening results.*

Phytochemical classes	Hydro-ethanolic extract	Aqueous extract
Favoids	+	+
Polyphenols	+++	++
Anthocyanins	++	-
Tannins	+++	+
Saponins	++	+++
Triterpenoids	++	+
Steroids	+++	+
Alkaloids	+	+

+: detected; ++: moderately concentrated; +++: highly concentrated -: not detected

4.3. Determining the Total Phenolic and Flavonoids Content of the Extract

4.3.1. Validation Parameters

Contents in phenolic compounds and flavonoids assessed subsequent to technique validation operation resulted in findings displayed in table 3.

Table 3. *Validation parameters for titration of total phenolic and flavonoids.*

Parameters	Polyphenol	Flavonoïds
Linearity equation	y = 0.0031x + 0.0008	y = 0.0112x + 0.0111
Determination coef (R^2)	0.9967	0.9978
LOD	1.121	0.738
LOQ	3.23	2.237
Accuracy (%CV)	3,47%	2.45
Slope sign (df = 13); α = 5%	T_{mes} (39.69) > $t_{0.025}$ (2.160)	T_{mes} (76.58) > $t_{0.025}$ (2.160)
Test y-intercept (df = 13) α = 5%	t(0,975) =-2.16 <t_{mes} =0.19<$t_{(0.025)}$ =2.160	t(0,975) =-2.16 <t_{mes} =1.15<$t_{(0.025)}$ =2.160
Precision	1.52% < Ref (2%)	1.09% < Ref (2%)

Data from this tables indicate a positive correlation between the amounts of analytes (phenolic compounds or

flavonoïds) and absorbance according to the correlation coefficient of 99.67% and 99.78%, respectively for phenolic contents and flavonoids contents. They also present for both metabolites, the compliance test of slope and intercept at a probability threshold α = 5% according to Student model. It can be observed from t-test analyses at 13 degree of freedom that the y-intercept is significantly different from 0 (0.19 and 1.15 both higher than -2.16) and that the slope is not different from 0 (39.69>2.160 and 76.58 > 2.160), respectively for phenols and flavonoïds.

4.3.2. Total Phenolic and Flavonoids Contents in Sample Extracts

Upon determination of the amounts of total phenols and flavonoid in specimens submitted according to the linearity equation pieces of information recorded were plotted as shown in figure 1.

Related overall findings indicate that the HE mixture (30-70) had the better extraction potential of phenolic compounds than the aqueous extract though the amounts of flavonoids appear to be approximately similar in both extracts.

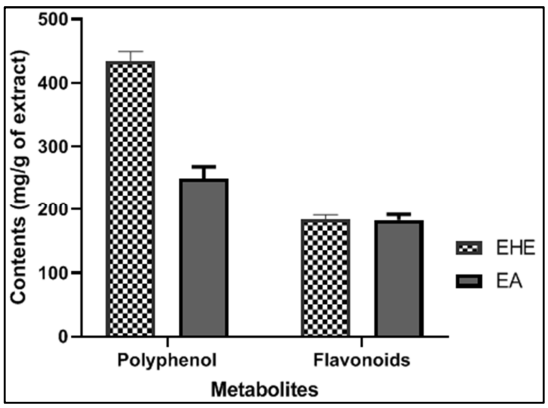

Figure 1. *Phenolic and flavonoid contents in aqueous extract (EAq) and hydroethanolic extract (HE) of Gardenia aqualla Staph: Each bar represents the mean ± SD; n=3.*

4.4. Assessing the Antibacterial of the Extracts

Susceptibility test conducted on subjected bacterial strains resulted in MIC MBC and MBC/MIC ratios values displayed in table 4 for both extracts.

Table 4. *MIC and MBC and MIC/MBC ratios values of crude extracts.*

Extracts	*Escherichia coli*			*Staphylococcus aureus*			*Klebsiella pneumoniae*			*Serratia marcescens*		
	MIC (mg/mL)	MBC (mg/mL)	$\frac{MBC}{MIC}$	MIC (mg/mL)	MBC (mg/mL)	$\frac{MBC}{MIC}$	MIC (mg/mL)	MBC (mg/mL)	$\frac{MBC}{MIC}$	MIC (mg/mL)	MBC (mg/mL)	$\frac{MBC}{MIC}$
AE	100	200	2	50	-	-	-					
HE	100	200	2	50	200	4	100	-	-	6.25	-	-

Related key pieces of information revealed that both extracts were active on the 2 strains of interest, with the lowest MIC (50 mg/mL) recorded on *Staphylococcus aureus*. Highest MIC values (100 mg/mL) were also obtained with both bacterial strains. Hydro-ethanolic extract showed a MIC of 100 mg/mL on *Klebsiella*. HE assays on *Serratia* showed a MIC of 6.25 mg/mL. These strains are known to be multi-resistant [35]. Unlike aqueous extract which presented for *E coli* a MBC of 200 mg/ml, hydro-ethanolic extract presented a MBC of 200 mg/mL on both strains of interest. No MBCs were not obtained on *Klebsiella and Serratia*, however the hydro-ethanolic extract of the leaves of *Gardenia aqualla* expressed bactericidal potential on E. coli and *Staphylococcus aureus*.

5. Discussion

This work targeted phytochemical study and antibacterial potential of aqueous and hydro-ethanolic extracts of fresh leaves of *Gardenia Aqualla* Staph harvested in Bangoua, West-Cameroon.

Ethno-pharmacological data from Cameroon indicated that the plant is used for the management of erectile dysfunction when macerated in palm or raffia wine. In Nigeria, Faleyimu et al (2012) reported that the plant was used alone in erectile dysfunction [35]. Raffia or palm wine is characterized by its contents in reducing sugar (about 7 g/L). accordingly, alcoholic fermentation could take place an hour post-harvest and give rise to 11% alcoholic solution [36, 37]. The extraction yields showed that hydro-ethanolic extract (6.11%) was higher than that of aqueous extract (4.33%). Hydro-ethanolic mixture (30:70) is known to be more polar solvent. Accordingly, active metabolites in the plant could be polar or weakly polar as observed by Penchev et al. (2010), on the effect of hydro-ethanolic solutions on polar compounds [38].

Phytochemical screening carried out on the both type of extracts revealed that hydro-ethanolic extract was richer in in investigated bioactive secondary metabolites particularly polyphenols, tannins, saponins and steroids, likely to be responsible for the antibacterial activity. In Nigeria, Njinga et al. (2014) detected in methanolic extract of the bark of the trunk of *Gardenia aqualla* Stapf & Hutch, the presence of steroids, triterpenoids, tannins, flavonoids, carbohydrates and petroleum. Ether extract from this plant revealed the presence of anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes [39, 40]. These metabolites confer to *Gardenia aqualla* set of properties including antioxydative [41, 42], antimicrobial [41, 43] anti-inflammatory and immunomodulatory activities [42].

These properties could be associated with the richness in in phenolic compounds which were more abundant in the hydro-ethanolic extract than in the aqueous fresh leaves extract during the present investigation. Almost similar in both extracts were flavonoids potential in the hydro-ethanolic extract, associated with the presence of others metabolites such as tannin, saponin terpenoids and steroids as described by Baan and Nazmul (2022) [43].

In vitro antibacterial assays revealed that hydro-ethanolic fresh leaves extract activity was effective on *Escherichia coli*

and *Staphylococcus aureus* with MIC values ranging from 50 through 100 mg/mL.

These results could contrast with those reported by Muhammad et al. (2019). These authors observed that the ethanolic extracts of *Gardenia aqualla* roots did not have antibacterial potential on the selected bacterial isolated except *E coli* [41]. The differences observed might be related to the geo-climatic and pedological characteristics of the area in which the plant grows. It may also be associated with the type of solvent and /or extraction technique used and even the part of the plant. In fact Muhammad et al (2019) assessed antioxidant, anti-inflammatory, antiproliferative and antimicrobial activities of *Combretum glutinosum* and *Gardenia aqualla* extracts in vitro used root ethanol extract.

BMC/MIC ratio for the hydro-ethanolic extract revealed bactericidal potential on 02 *E. coli* and *Staphylococcus aureus* while; the BMC/MIC value for the aqueous extract was bactericidal on the *E. coli* and not *S. aureus*. These findings could be justified, at least partially by the chemical composition of both extracts. In fact, polyphenols are known to have the ability to combine with soluble extracellular proteins of the bacterial cell walls and cause their lysis [44, 45]. Therefore, it can be hypothesized that the hydro-ethanolic extract contains more other polyhydroxylated compounds such as saponin than the aqueous extract which can react with bacteria, providing explanation the activity of EHE on *Serratia marcescens*. The results also indicated that *Serratia marcescens* was only partially susceptible with MIC = 6.25.

6. Conclusion

The aim of this work was to determine the chemical composition of aqueous and hydro-ethanolic extract of fresh leaves of *Gardenia aqualla Staph & Hutch* and to assess the antibacterial potential on multiresistant isolates involved in erectile dysfunction. Qualitative tests showed the presence of alkaloids in both AE and HE, phenolic compounds including tannins, flavonoids with good levels in HE than in AE and anthocyanin absent in AE. Saponins was more abundant in AE while steroid and terpenoids was more in HE. Quantitative phytochemical tests showed the presence of great amount of phenolic compounds in HE than in aqueous extract with a slightly equivalence in amount of flavonoids. The main strains of interest (*E. coli* and *S. aureus*) were susceptible to both extract despite HE had a better activity extended to *Klebsiella* and *Serratia marcescens* which are associated with acute and chronic epididymitis, prostatitis.

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