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Phytochemical screening, antioxidant, anti-biofilm and antifungal activities of aqueous and ethanolic extracts of *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* in Benin

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Key words: *Euphorbia hirta*, *Citrus aurantifolia, Heterotis rotundifolia*, Antioxidant, Anti-biofilm, Antifungal.

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Abstract

Euphorbia hirta, *Citrus aurantifolia* and *Heterotis rotundifolia* are plants of the traditional Beninese medicine. They are used in the treatment of several diseases, such as diarrheal infections, salmonellosis, malaria and others. This study aims to contribute to the scientific knowledge of these plants through their phytochemical screening as well as the *in vitro* evaluation of the antioxidant, antibiofilm and antifungal activity of their aqueous and ethanolic extracts. The phytochemical screening of these plants was evaluated by the standard method. The antioxidant activity was determined through the DPPH radical and the FRAP method. Anti-biofilm and antifungal activities were performed using the colorimetric method and the mycelial growth inhibition method respectively. Phytochemical screening of these plants revealed the presence of secondary metabolites such as: tannins, flavonoids, anthocyanins, quinonic derivatives etc. The aqueous and ethanolic extracts of these plants have antioxidant properties with IC50s ranging from 23.7 to 55.36 µg/ml (DPPH) and 0.43 to 1.96 mg/ml (FRAP). The anti-biofilm and antifungal effects of these extracts vary according to strains, extracts and plants. The inhibition percentages of bacterial biofilm are between 20.66 and 81%. The highest percentage of mycelial growth inhibition is 69.23±1.86% obtained with the ethanolic extract of *Euphorbia hirta* on *Fusarium solani*. The antioxidant, anti-biofilm and antifungal activities are much more remarkable with ethanolic extracts. These different results may justify the use of these plants in traditional medicine. *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* are therefore of medicinal interest as a potential source of natural bioactive substances.

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Introduction

Nowadays, extraordinary therapeutic virtues are attributed to plants. Their use for the treatment of several diseases in living beings and in particular in humans is very old and has always been done empirically (Nacoulma, 1996; Svoboda et Svoboda, 2000). Studies have shown that the active ingredients of medicinal plants are often linked to the products of secondary metabolites. Their properties are currently recognized, listed and used in traditional and modern medicine (Bourgaud *et al.,* 2001; Kar, 2007). Currently, it is estimated that 25% of the drugs prescribed are derived from plants active ingredients. Be a total of 120 compounds of natural origin from 90 different plants. According to some estimates, more than 13,000 species of medicinal plants are used as traditional medicines by various cultures around the world (Tyler, 1993). In Africa, nearly 6377 plant species are used, of*ET AL*which more than 400 are medicinal plants that contribute 90% of the medical treatment (Kar, 2007). A study by the World Health Organization (WHO) also found that eight out of ten Africans (80%) use medicinal plants to their basic health care (WHO, 2002).

In addition, free radicals are said to cause several diseases, including osteoarthritis, asthma, cancer, diabetes, heart disease, atherosclerosis etc. (Codoñer-Franch *et al.,* 2011; Sarr *et al.,* 2015). The use of synthetic antioxidant molecules in the fight against these free radicals is currently being questioned due to potential toxicological risks. New plant sources of natural antioxidants are from now being sought (Suhaj, 2006; Tadhani *et al.,* 2007).

Indeed, various bacteria and fungi are directly or indirectly involved in human infections. Despite the damage caused by these pathogens, the scientific community has discovered many treatments to relieve patients. But, due to the ever-increasing cost of available drug prices combined with the ongoing resistance of several bacterial and fungal strains, there is a renewed interest in pharmacopoeia (Akoua *et al.,* 2004, Guillemot *et al.,* 2004; Millogo-Koné *et al.,* 2008). Thus, the use of natural substances extracted from plants could constitute a serious alternative to chemical antibiotics and fungicides.

Among the plants widely used in traditional pharmacopoeia in Benin but poorly studied scientifically, are *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* (Dougnon *et al.,* 2017). This work was undertaken with the aim of contributing to the scientific knowledge of these plants through identification of the secondary metabolites present therein and in vitro evaluation of the antioxidant, anti-biofilm and antifungal activities of their extracts.

Materials and methods

Plants collection and identification

Whole plants of *Euphorbia hirta* (*E. hirta*) and *Heterotis rotundifolia* (*H. rotundifolia*) as well as *Citrus aurantifolia* (*C. aurantifolia*) leaves were used. These plants were collected in Ouidah (*E. hirta*) and Abomey-Calavi (*C. aurantifolia* and *H. rotundifolia*) and then confirmed at the national herbarium of the University of Abomey-Calavi. They were then washed and dried in the laboratory at 25°C for two weeks. After drying, they were powdered. The powders were used to perform phytochemical screening and were also used to prepare aqueous and ethanolic extracts.

Microbial strains

The anti-biofilm activity of the extracts was evaluated on four biofilm-producing strains, namely: *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Salmonella choleraesuis* (*S. choleraesuis*) and *Shigella flexneri* (*Sh. flexneri*). The antifungal activity was evaluated on five fungal strains, namely: *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*), *Penicillium cyclopium* (*P. cyclopium*), *Penicillium expansium* (*P. expansium*) and *Fusarium solani* (*F. solani*). These strains are part of the collection of the Laboratory of Biology and Molecular Typing in Microbiology.

Phytochemical screening

The phytochemical screening performed is a qualitative chemical analysis based on colouring or

precipitation reactions. The method used is that of Houghton and Raman (1998) adapted to laboratory conditions.

Preparation of aqueous extracts

It is a 10% extraction which was carried out by macerating 50 g of plant powder in 500 ml of distilled water under magnetic agitation for 48 hours at room temperature. After 2 successive filtrations on hydrophilic cotton and once on Whatman N°1 paper, the filtrate was dried at 50°C in an oven and the powder thus obtained constitutes the total aqueous extract (Guede-Guina *et al.,* 1995).

Preparation of ethanolic extracts

The ethanolic extract was obtained in the same way as the aqueous extract except that the filtrate was concentrated with rotavapor under vacuum at 50°C before being dried in the oven. 96% ethanol was used (Sanogo *et al.,* 2006; N'Guessan *et al.,* 2007).

Extraction yield

Extraction yield is defined as the ratio between the mass of the dry extract obtained and the mass of the treated plant material (Harborne, 1998). It has been obtained according to the following formula:

$$Y(\%) = \frac{Me}{Mp} \times 100$$

Y (%): Yield in %; Me: Mass of dry extract, Mp: Mass of plant material used.

Evaluation of the antioxidant activity of plant extracts

The antioxidant activity of the aqueous and ethanolic extracts of the plants studied was evaluated following two methodologies: the scavenging method of the free radical DPPH (2,2-diphenyl picryl-hydrazyl) and the FRAP (Ferric Reducing Antioxidant Power) test with determination of IC50 (Concentration that inhibits 50% of DPPH or reduces 50% of Fe^{3+}) from the percentages of DPPH inhibition or Fe^{3+} reduction.

DPPH test

The anti-free radical activity of the various extracts was evaluated according to the method described by Mansouri *et al.* (2005). This method uses DPPH (2,2-diphenyl picryl-hydrazyl) as a relatively stable free radical. Antioxidants reduce purple diphenyl picryl-hydrazyl to a yellow compound, whose color intensity is inversely proportional to the ability of antioxidants in the medium to give protons (Sanchez-Moreno, 2002). Thus, from 400 µg/ml of extract solution, a series of 10 successive dilutions (at 1/2) of each extract is carried out with methanol in order to have 1 ml volume. Then, 1 ml of DPPH (40 µg/ml in methanol) is added to all cascade dilutions. At the same time, a negative control is prepared by mixing 1 ml of methanol with 1 ml of the methanol solution of DPPH. The absorbance reading is made against a blank prepared for each concentration at 517nm after 20 min incubation in the dark and at room temperature.

The positive control was made by a standard antioxidant solution which is the vitamine C whose absorbance has been measured under the same conditions as the samples and for each concentration, the test was repeated 3 times. The inhibition percentage of DPPH radical was calculated according to the following formula:

$$IP(DPPH) = \frac{Abs(blank) - Abs(extract)}{Abs(blank)} \times 100$$

IP (DPPH) = Inhibition percentage of DPPH; Abs=Absorbance

The IC50 (Concentration that inhibits 50% of DPPH) was determined by the probits method from a regression of inhibition percentages as a function of extract concentrations.

FRAP test

The reducing power of the extracts was determined by FRAP (Ferric Reducing antioxidant Power) method according to the protocol described by Dieng *et al.* (2017). Thus, 0.5 ml of sample (extract) at different concentrations is mixed with 1 ml of phosphate buffer (0.2 M; pH=6.6) and 1 ml of 1% potassium hexacyanoferrate[$K_3Fe(CN)_6$]. After mixture

incubation at 50°C for 30 minutes, 1ml of 10% trichloroacetic acid was added to each tube to stop the reaction. The tubes are then centrifuged at 3000 rpm for 10 minutes. 1 ml of the supernatant from each tube was mixed with 0.2 ml of a 0.1% $FeCl_3$ solution and left to stand in the dark for 30 minutes. The absorbances were measured at 700 nm against a blank prepared under the same conditions and without extract. The reducing power of the extracts has been expressed as a percentage according to the formula:

$$RP = \frac{Abs(extract) - Abs(blank)}{Abs(extract)} \times 100$$

RP= Reducing Power; Abs=Absorbance

The IC_{50} (Concentration that reduce 50% of Fe^{3+}) was determined by the probits method from a regression of reducing power (percentages) as a function of extract concentrations.

Evaluation of the anti-biofilm activity of extracts

The effect of extracts (aqueous and ethanolic) on the biofilm formation of four microbial strains was evaluated in 48-well flat-bottomed polystyrene plates. It followed the protocol of Nikolić *et al.* (2014) with some modifications. Thus, 200 µL of MH broth containing 10^8 CFU of each microbial strain was distributed to each well of the microplate. Then 200 µL of extract at 100 mg/ml was added to each well. Wells containing medium with strains and without extracts were used as controls. The plates were incubated at 37°C for 48 hours. After incubation, the supernatant was removed and each well was thoroughly washed with sterile distilled water to remove floating cells. The plates were then dried at room temperature for 30 min. 200 µL of 0.1% aqueous crystal violet solution was added to each well to colour any biofilm formed for 15 min at room temperature. After incubation, the excess dye was removed by washing the plate three times with sterile distilled water. Finally, the dye bound to the microbial biofilm formation was solubilized by adding 95% ethanol to each well and after 15 minutes of incubation, the absorbance was measured at 570 nm (Nikolić *et al.*, 2014; Tutar *et al.*, 2016). The biofilm inhibition percentage (BIP) was calculated for each strain using the formula:

$$BIP = \frac{Abs(contrôle) - Abs(test)}{Abs(contrôle)} \times 100$$

BIP=Biofilm Inhibition Percentage; Abs=Absorbance

Antifungal activity of extracts

The antifungal activity of the plant extracts was evaluated by a method inspired by that described by Karanga *et al.* (2017). Thus, an extract solution prepared in distilled water was added to the Potato Dextrose Agar (Agar PDA) in order to have an extract concentration of 100 mg/ml. The whole thing was sterilized in an autoclave at 121°C for 15 minutes and then poured into a 90 cm petri dish. After solidification, a disc of Whatman paper (6 mm diameter) contaminated on one of its surfaces by the pathogen (fungal strain) was deposited in the center of the petri dish containing the medium supplemented with extract. A control was carried out in the same condition with the medium without extract. Each test was performed three times. The petri dishes were incubated at 25°C. After 5 days of incubation the mycelium diameter was measured for each fungal strain and the percentage of mycelium inhibition was calculated according to the following formula:

$$MIP = \frac{MD(contrôle) - MD(test))}{MD(contrôle)} \times 100$$

MIP= Mycelium Inhibition Percentage; MD= Mycelium Diameter

Data analysis and processing

GraphPad Prism 7 software was used to perform graphs and statistical analyzes. A comparison of mean was made with analysis of variances (ANOVA) followed by student test. The differences are considered significant if p-value < 0.05 and very significant if p-value < 0.001.

Results

Table 1 presents the result of the phytochemical screening carried out with the powders of the plants studied. It should be noted that the three plants do

not have the same phytochemical profile. Indeed, the presence of tannins (gallic and catechic tannins), flavonoids, anthocyanins, quinonic derivatives, triterpenoids, mucilages and saponosides is noted in all plants. However, none of the plants contain cardenoids, cyanogenic derivatives, O-Heteroside, Reduced-genin O-Heterosides and CHeterosides.

Table 1. Secondary metabolites present in plants (*Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia*) studied.

Metabolites sought	*Euphorbia hirta*	*Citrus aurantifolia*	*Heterotis rotundifolia*
Composé azoté			
Alkaloids	-	-	+
Polyphenolic compounds			
Tannins	+	+	+
Catechic tannins	+	+	+
Gallic tannins	+	+	+
Flavonoids	+	+	
Anthocyanins	+	+	+
Leuco-anthocyanins	+	-	+
Coumarins	+	-	-
Quinonic derivatives	+	+	+
Terpene compounds			
Triterpenoids	+	+	+
Steroids	-	+	-
Cardenoids	-	-	-
Heterosides			
Cyanogenic derivatives	-	-	-
Mucilages	+	+	+
Saponosides	+ (MI 136)	+ (MI 178)	+ (MI 201)
Reducing compounds	+	+	-
Free Anthracene	-	+	+
O-heterosides	-	-	-
Reduced-genin O-Heteroside	-	-	-
C- heterosides	-	-	-

+ : Presence ; - : Absence ; MI : Moss Index.

If the screening revealed the presence of alkaloids in *H. rotundifolia*, it's opposite in *E. hirta* and *C. aurantifolia*. leuco-anthocyanins were detected only in *E. hirta* and *H. rotundifolia*. As well, steroids and free anthracene were revealed only in *C. aurantifolia* (Table 1). Fig. 1 shows the extraction yield of each plant. It can be seen that the extraction yields of ethanol extracts are higher than those of aqueous extracts. Ethanol has therefore better concentrated the secondary metabolites of these plants than water.

In addition, the antioxidant activity of these plants extracts was evaluated through the DPPH radical and the FRAP method. The results are showed in Fig. 2.

It can be seen that the IC_{50s} of ethanol extracts are lower than those of aqueous extracts for each plant. However, more the IC_{50} is lower, more the antioxidant power is higher. Ethanolic extracts therefore have a higher antioxidant power than aqueous extracts. Concerning the anti-free radical

power through the DPPH radical, the IC50s vary from 23.7 (Ethanolic extract of *E. hirta*) to 55.36 (Aqueous extract of *H. rotundifolia*) µg/ml. There is a very significant difference ($p<0.001$) between the IC_{50s} of the aqueous and ethanolic extracts of *E. hirta* and *C. aurantifolia* while at the *H. rotundifolia* level, there is no significant difference ($p>0.05$) between the IC_{50s} of these two extracts (Fig. 2a). In terms of reducing powers, the IC50s range from 0.43 (Ethanolic extract *H. rotundifolia*) to 1.96 (Aqueous extract of *E. hirta*) mg/ml. There is a significant difference ($p<0.05$) between the IC50s of the aqueous and ethanolic extracts of *E. hirta* and *H. rotundifolia* while at the *C. aurantifolia* level, there is no significant difference ($p>0.05$) between the IC50s of these two extracts (Fig. 2b). It should also be noted that at the level of extracts as well as at the level of the reference molecule (Vitamin C) used as standard, the IC_{50} of the anti-radical powers are much lower than the IC_{50} of the reducing powers (Fig. 2 a and b).

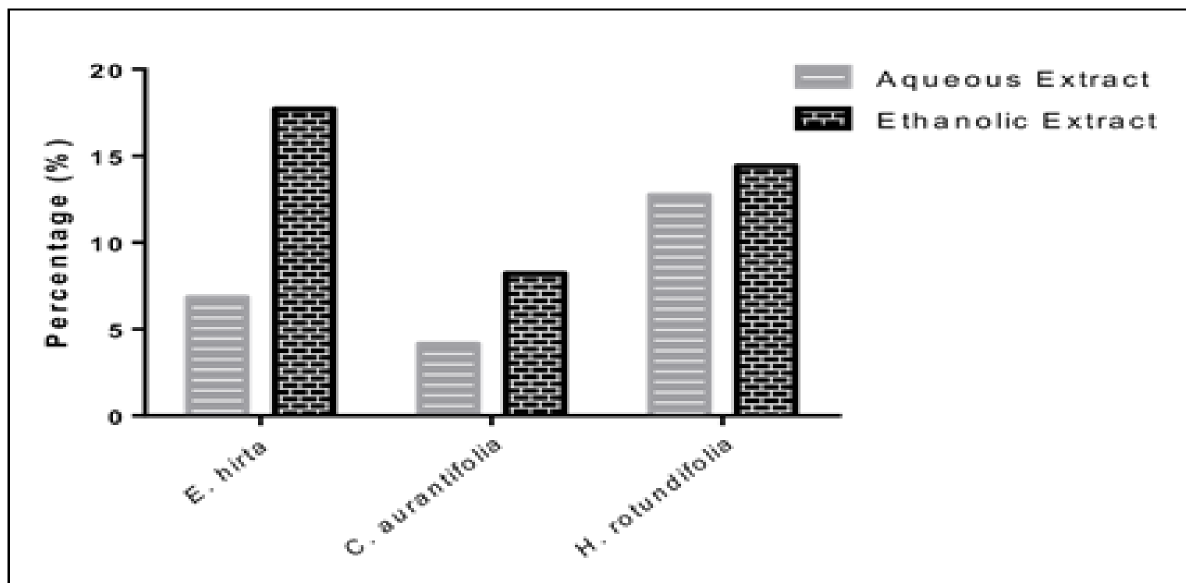

Fig. 1. Yield of Extracts.

Fig. 3 shows the percentages of biofilm inhibition by the two types of plant extracts studied. It can be seen that the percentages of biofilm inhibition vary according to strains, extracts and plants. Both types of *E. hirta* extracts have no effect on the microbial biofilm of *K. pneumoniae*. The lowest percentage of inhibition (20.66%) was obtained with ethanolic extract and the highest percentage of inhibition (81%) was obtained with aqueous extract on microbial biofilm of *Sh. flexneri* and *S. choleraesuis* respectively (Fig. 3a). As with *E. hirta* extracts, *C. aurantifolia* extracts have no effect on the microbial biofilm of *K. pneumoniae*. The lowest percentage of inhibition (23.66%) and the highest percentage of inhibition (68.33%) were obtained with the ethanol extract of this plant on the microbial biofilm of *Sh. flexneri* and *E. coli* respectively (Fig. 3b). At the *H. rotundifolia* extracts level, the lowest percentage of inhibition (25%) and the highest percentage of inhibition (73.66%) were obtained with ethanol extract on the microbial biofilm of *K. pneumoniae* and *Sh. flexneri* respectively (Fig. 3c).

In addition, Fig. 3 analysis shows that ethanolic extracts have more effect on the biofilm formation of tested strains than aqueous extracts, except for *H. rotundifolia* extracts. The inhibition percentages of biofilm are between 20.66 and 81% for all extracts.

Fig. 4 shows the percentages of inhibition of mycelial growth of fungal strains by the two types of extracts of the three plants studied. It can be seen that the inhibition percentages vary according to extracts, plants and strains. For *E. hirta*, both types of extracts of this plant have inhibited the mycelial growth of all fungal strains tested and the inhibition percentages of

ethanol extracts are higher than those of aqueous extracts. The lowest inhibition percentage is 22.46±0.92% obtained with the aqueous extract on *P. expansium* and the highest inhibition percentage is 69.23±1.86 obtained with the ethanolic extract on *F. solani* (Fig. 4a). Concerning *C. aurantifolia* extracts, it is noted that the aqueous extract of this plant has no effect on the mycelial growth of *A. niger* and *P. cyclopium* while the ethanolic extract of this plant has inhibited the mycelial growth of all fungal strains with varying percentages.

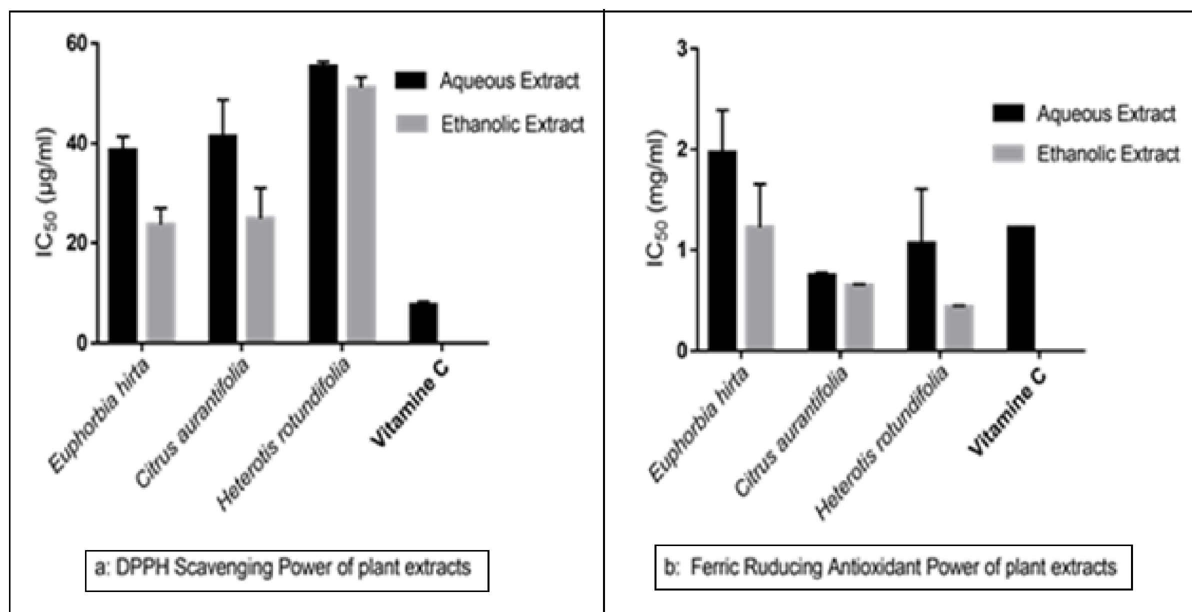

Fig. 2. *In Vitro* antioxydant activity of plant extracts.

The lowest percentage of inhibition (27±2.84%) and the highest percentage of inhibition (66.18±1.71%) was obtained with the ethanolic extract of this plant on *P. cyclopium* and *A. flavus* respectively (Fig. 4b). Finally, for *H. rotundifolia*, the two types of extracts of this plant inhibited the mycelial growth of the two *Aspergillus* species, whereas these two extracts have no effect on *Penicillium* species. The lowest percentage of inhibition obtained with extracts of this plant is 21.84±1.51% (ethanolic extract) on *F. solani* and the highest percentage of inhibition is 50.70±2.43% (ethanolic extract) on *A. flavus* (Fig. 4c).

Figures 1,2,3 and 4 legends: E. hirta: Euphorbia hirta, C. aurantifolia: Citrus aurantifolia, H. rotundifolia: Heterotis rotundifolia, Vit: CVitamine C, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, S. choleraesuis: Salmonella choleraesuis, Sh. Flexneri: Shigella flexneri, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. cyclopium: Penicillium cyclopium P. expansium: Penicillium expansium, F. solani: Fusarium solani

Discussion

The biological activities attributed to medicinal plants are often linked to the secondary metabolites they contain. The highlighting of the different classes of secondary metabolites in the three plants (*Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia*) studied, allows us to have a good idea on their pharmacological activities.

In this study, the secondary metabolites sought varied according to plant. Nevertheless, tannins, flavonoids and saponosides were revealed in all the plants studied. These three secondary metabolites had already been revealed in these plants extracts by Ayéna *et al.* (2017), Fasola and Iyamah (2014) and Balogun and Owoseni (2013) respectively for *E. hirta*, *C. aurantifolia* and *H. rotundifolia*. In our results, we note the absence of alkaloids in *E. hirta* and *C.*

aurantifolia. This result is contrary to those of Chitra *et al.* (2011) and Al Namani *et al.* (2018) who noted the presence of alkaloids in *E. hirta* and *C. aurantifolia* extracts respectively. Similarly, in this study, reducing compounds were absent in the phytochemical screening of *H. rotundifolia*. On the other hand, Adinortey (2015) in his study had revealed the presence of this group of chemical compound in this plant. The difference observed in this study and those of the above-mentioned authors could be explained by several factors such as soil type, plant harvesting season and plant harvesting location.

However, the absence of cyanogenic derivatives in these plants may reduce the risk of toxicity associated with their use. Indeed, cyanogenic derivatives can cause intoxication through cyanide ion production whose ingestion can induce accelerated heart rate, dizziness, headaches, consciousness disorders etc. (Lagnika *et al.,* 2011).

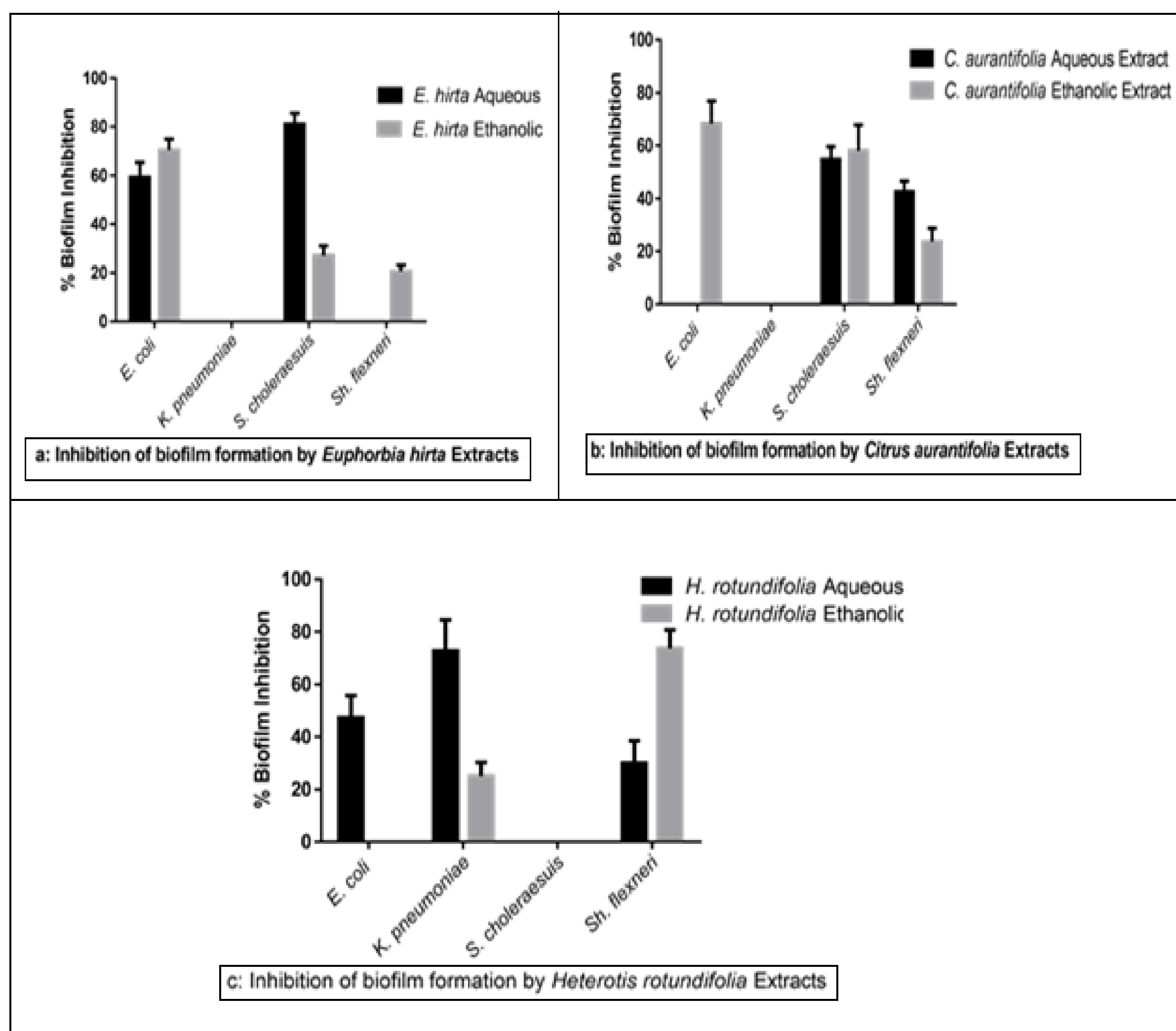

Fig. 3. Inhibition of biofilm formation by plant Extracts.

Concerning antioxidant activity, the results obtained showed that the ethanolic extracts of the three plants (*E. hirta*, *C. aurantifolia* and *H. rotundifolia*), are more active than the aqueous extracts with lower IC50s for both tests performed. This could be explained by the fact that ethanol has better concentrated the active ingredients of these plants than water. In addition, the antioxidant activity of plant extracts is often attributed to secondary metabolites, particularly flavonoids and polyphenols contained in them. Indeed, polyphenols are known as antioxidant substances with the ability to trap radical species and reactive forms of oxygen (Kelly *et al.,* 2002). Many studies have also established

relationships between the chemical structures of flavonoids and their antioxidant capacity (Amic *et al.,* 2003). However, polyphenols and flavonoids have been identified in these plants extracts (Dougnon *et al.,* 2017). As well, the effect of the two types of extracts from the three plants studied was evaluated on the biofilm formation of four bacterial strains (*E. coli*, *K. pneumoniae*, S. *choleraesuis* and *Sh. flexneri*). This follows an evaluation of the antibacterial activity of these extracts in another study (Dougnon *et al.,* 2017).

Bacterial biofilms are structured clusters of bacterial cells coated with a polymeric matrix and attached to a surface. Biofilm protects bacteria and allows them to survive in harsh environmental conditions.

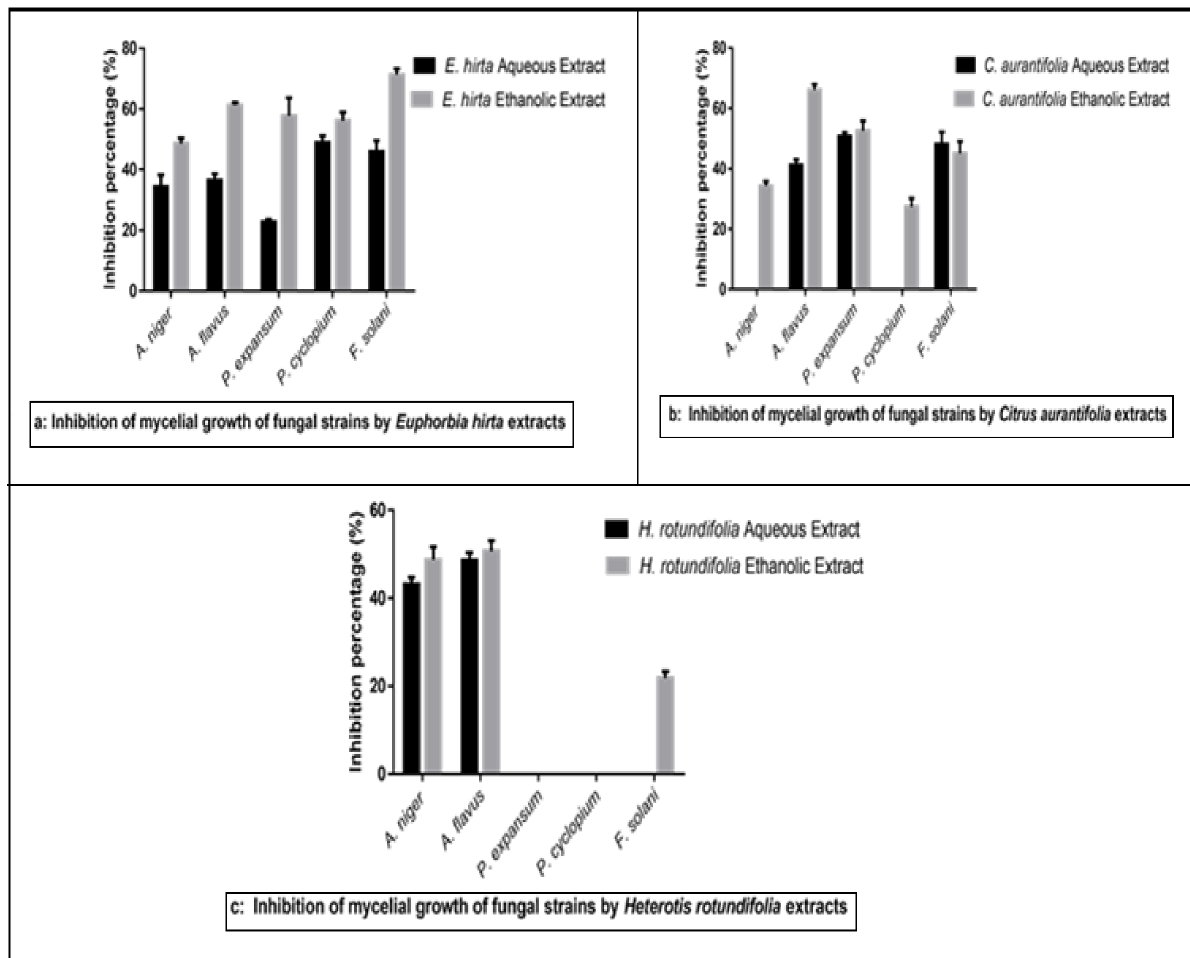

Fig. 4. Inhibition of mycelial growth fungal strains by plant extracts.

The bacteria in the biofilm are physiologically different and are much more resistant not only to antibiotics but also to the host's immune response than planktonic bacteria (Tremblay *et al.*, 2014). In this study, the plant extracts studied inhibited the biofilm formation of the strains tested with inhibition percentages ranging from 20.66 to 81%. These percentages are similar to those of Tutar *et al.* (2016) in their study on the antibiofilm and antimicrobial activity of *Mentha pulegium* L essential oil against multiresistant strains of *Acinetobacter baumannii* at different concentrations. The antibiofilm effect observed with these plants extracts may be related to the action of the flavonoids contained in these extracts (Dougnon *et al.,* 2017).

Indeed, previous studies have reported that molecules from plants belonging to the flavonoid family (quercetin, naringenin, kaempferol, etc.) are able to reduce bacteria biofilm production by suppressing auto-inducer 2 activity, responsible for intercellular communication (Vikram *et al.,* 2010).

The antifungal activity carried out with the extracts (aqueous and ethanolic) of our plants shows that these extracts have an antagonistic effect on the mycelial growth of the strains tested and the inhibition percentages vary according to the strains, extracts and plants. The antifungal effect of *E. hirta* had already been demonstrated in several studies such as that of Ndam *et al.* (2018) and Karanga *et al.* (2017) with different inhibition percentages than ours. The difference observed at this level can be explained by the difference of the strains, the extraction methods used and the concentrations of extracts tested. The aqueous extract of *C. aurantifolia* has no effect on the mycelial growth of *A. niger*.

This result is contrary to that of Nweke (2015) who found that the aqueous extract of this plant inhibits the proliferation of this strain. However, our results are similar to those of Ezikanyi *et al.* (2016) who found that the ethanolic extracts of *C. aurantifolia* inhibit the proliferation of several fungal strains including *Aspergillus sp.*, *Candida sp.* and *Penicillium sp.* with concentrations ranging from 75 to 300 mg/ml. In other wise, *H. rotundifolia* extracts did not inhibit the mycelial growth of *Penicillium* strains. Indeed, the antifungal activity of this plant is fewly reported in previous studies.

Conclusion

This study shows that the aqueous and ethanolic extracts of *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* have interesting and variable antioxidant activities. The highlighting of anti-biofilm and antifungal activities has shown that these extracts inhibit the biofilm formation and mycelial growth of the strains tested with inhibition percentages that vary according to the strains, extracts and plants. These different biological activities can be attributed to secondary metabolites (tannins, flavonoids, anthocyanins, quinonic derivatives etc.) revealed by phytochemical screening.

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