Chemistry Research Journal, 2021, 6(2):137-146

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Phytochemical screening, antioxidant capacity, antibacterial and anti-inflammatory activities of ethanolic extract of *Cordia senegalensis* leaves, a plant used in Benin to treat skin diseases

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Abstract Natural substances extracted from the vegetal biomass have multiple benefits exploited in industrial biotechnology of food, cosmetics and pharmaceuticals as well. The present study reports the phytochemical analysis and antibacterial activity of leaves which are widely used in traditional medicine to treat skin diseases. Secondary metabolites were identified by coloration and precipitation reactions specific to each family of metabolites. The determination of total phenolic compounds was made by Folin-Ciocalteu reagent. The aluminum trichloride method has been used to quantify total flavonoids, while the determination of condensed tannins was carried out by the hydrochloric vanillin method. The antioxidant capacity of ethanolic extract of *Cordia senegalensis* leaves was evaluated by the DPPH, ABTS and phosphomolybdenum method. The antibacterial activity was evaluated in microplates and in Petri dishes. According to the results obtained, the leaves of *Cordia senegalensis* contain flavonoids, anthocyanins, leuco-anthocyanins, mucilages, terpenes and sterols. The contents of total phenolic (53.81±0.84) µgGAE/mgDM, total flavonoids (12.81±0.01)µgQE/mgDM and condensed tannins (1.463±0.375) µgCE/mg DM are very interesting. The results obtained during this work allowed us to assert that all extracts of the studied plant have very good antioxidant properties. The ethanolic and hydroethanolic extracts of *Cordia senegalensis* inhibited only the strains of *Staphylococcus aureus*, *Staphylococcus epidermidis* at a concentration of 10mg/mL with an interesting anti-inflammatory activity (91.390±0.011)% more than aspirin (38.450±0.120)%. The ethanolic extract of *Cordia senegalensis* could be used to fight against free radical attacks on the skin, to treat skin conditions and for the preservation of perishable food products.

Keywords *Cordia senegalensis*, secondary metabolites, antibacterial, antioxidant, dermatosis, anti-inflammatory

Introduction

For millennia, all people have developed medicines according to their intelligence, genius, cultural conception of health, disease and the relationship they have with their environment [1]. Most plants species that grow all over the world have therapeutic properties, because they contain active ingredients that act directly on the body [2]. Despite the progress of modern medicine, ancestral therapeutic traditions are perpetuate in Africa where more than 80% of the population continues to use medicine traditional to heal. This widespread use is explained by accessibility and availability of traditional medicine in developing countries on one hand, as well as the high cost and harmfulness of the side effects caused by synthetic drugs on the other hand. All these observations justify all the actions currently being carried out with a view to developing traditional medicine and ensuring its integration into modern national health care systems. Many plants species contain secondary metabolites with the potential to combat disease causing micro-organisms. These compounds include glycosides, saponins, flavonoids steroids, tanins, alkaloids and terpenes [3]. The antioxidant potential of plants has been found to be a promising method of countering the undesirable effects of oxidative stress. Constantly exposed to external aggressions, the skin represents a privileged target of oxidative stress, which leads to multiple skin damage [4]. Antibiotics widely used for the treatment of infectious diseases are under constant threat due to the emergence of resistant and multi-resistant pathogens antibiotics [5] [6]. This resistance to antibiotics in microorganisms pathogens cause premature death and are a problem more and more important in the world [7]. Many cases of multidrug resistance have been reported in sub-Saharan Africa [8]. *Staphylococcus aureus* is responsible for endocarditis and secondary wound infections and also for several skin infections [9]. Skin health still remains a topical and public health issue in a context where the skin is subject to numerous external aggressions [10]. *Cordia senegalensis* of the Boraginaceae family is a shrub or tree reaching 7 to 8 meters in height. *Cordia senegalensis* is one of the plants most used in traditional medicine in Benin to treat skin diseases. The deepening of scientific studies of *Cordia senegalensis* therefore becomes a priority in order to optimize its use in traditional medicine. This study focused on the phytochemical, antioxidant, antimicrobial, and antiinflammatory properties of the *Cordia senegalensis* medicinal plant.

Materials and Methods

Materials

Plant material: *Cordia senegalensis* leaves used in this study were harvested in the Couffo in Benin.

Chemicals: Methanol, Folin–Ciocalteu reagent, 2,2-diphenyl-1- picrylhydrazyl (DPPH), quercetin, gallic acid, aluminum chloride, potassium acetate, sodium acetate, ascorbic acid, aspirin, catechin, trolox, ammonium molybdate, ascorbic acid, hydrochloric acid, sulfuric acid and 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) were purchased from Sigma-Aldrich. All reagents and chemicals were analytical grade.

Bacterial material: The bacteria strains used are provided by the Biochemistry and Molecular Biology Unit of the University of Abomey-Calavi. These are the reference strain of *Staphylococcus aureus* ATCC 6538 and clinical strain of *Staphylococcus epidermidis.*

Methods

***Cordia senegalensis* leaf pretreatment:** After harvesting, the samples were dried at laboratory temperature until their plant mass stabilized and then reduced to powder.

Plant extracts: The extraction was made with ethanol and hydroethanolic under ultrasounds. Briefly, 10 g of powdered biomass were mixed with 100 mL solvent and sonicated for two hours at 50℃ with Bandelin (Sonorex Digitech device). Further, all the extracts were filtered through Whatman No.1 filter paper and concentrated under vacuum (Buchi R215, heating bath B-491, rotation 280 rpm, vacuum controller V-850 of 290 mbar) at 50℃±1℃. The residues were dried to constant weights and stored in the darkness at 4℃ to avoid the degradations until use [11-13].

Preliminary phytochemical screening

Secondary metabolites were carried out by coloration and precipitation reactions specific to each family of metabolites [14-16].

Determination of polyphenolic compounds

Total phenol content: The total phenolic content of the various extracts was quantified by using the Folin–Ciocalteu reagent. This method consisted of using a mixture of phosphotungstic and phosphomolybdic acids, which were reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum. Finally, the absorbance was measured at 760 nm using a spectrophotometer (with Infinite 200 PRO-Tecan microplate) and the total phenol content are expressed in micrograms of gallic acid equivalence per milligram of dry matter (µgGAE/mgDM) [11, 17-18].

Total flavonoids content

The method of aluminum trichloride ($AlCl_3$) was used to quantify the total flavonoids. This technique was based on the formation of the aluminum complex flavonoids. The absorbance was read at 415 nm using a spectrophotometer (Infinite 200 PRO-Tecan microplate) and the Total flavonoid content are expressed in micrograms quercetin equivalence per milligram of dry matter (µgQE/mgDM) [19].

Condensed tannin content

The condensed tannins dosing was achieved by the method of hydrochloric vanillin. The absorbance were measured at 500 nm using the spectrophotometer (Infinite 200 PRO-Tecan microplate) and the tannin content was expressed in micrograms catechin equivalence per milligram dry matter (µgEC/mgDM) [20].

Antioxidant activity

The Antioxidant activity presented by the *Cordia senegalensis* extract was quite different from that measured by the ABTS, DPPH and ammonium molybdate test.

DPPH free radical scavenging assay

100 µL of ethanolic extract of *Cordia senegalensis* leaves were added to the five wells of the first two 96-well microplate lines. Then a gradual dilution of a ratio of two was carried out starting with the second line with methanol until the last line (8th line) where 100 µL of dilution was discarded at each well. In the first three wells of each row, 100 µL of the methanolic solution of DPPH (0.1mg/mL) were added and 100µL of methanol in the other, two wells of each row to prepare the negative control. The positive control is prepared in parallel by mixing 100µL of methanol with 100µL of the DPPH solution. After incubation in the dark room the temperature, the absorbance is measured after 15 minutes for one hour at 517 nm using the spectrophotometer (microplaque Infinite 200 PRO-Tecan) [11, 18, 21].

$$P(\%) = \frac{[A_{posi\ \ contr} - (A_{sam} - A_{nega\ \ contr})]}{A_{posi\ \ contr}} X100$$

$A_{posi\ contr}$: Positive control absorbance; $A_{contr\ negati}$: Negative control absorbance ; A_{sam}: sample absorbance

Ammonium molybdate test

100µl of ethanolic extract of *Cordia senegalensis* at different concentrations were added to 1000µL of a reagent composed of sulfuric acid (0.6M), sodium phosphate (28mM) and ammonium molybdate (4mM). The tube was incubated at 95°C for 90 minutes and after cooling, the absorbance was measured at 695nm. The control consists of 100µL of dissolution solvent mixed with 1000µL of the reagent mentioned above. Samples and controls are incubated under the same conditions and then the absorbance is measured using a spectrophotometer (Infinite 200 PRO-Tecan). The results obtained are expressed in micrograms ascorbic acid equivalent per milligram of dry matter of the extract (µgAAE/mgDM) [22].

ABTS free radical scavenging assay

The potential of the extracts to reduce the $ABTS^{\cdot+}$ radical was evaluated in using the method described by Miller *et al* [23]. The principle is based on the ability of an antioxidant to stabilize the blue-green cationic radical $ABTS^{\cdot+}$ by transforming it into colorless $ABTS^{\cdot+}$ by trapping a proton. The cationic radical $ABTS^{\cdot+}$ was obtained from 10 ml of ABTS (2mM) and 100 µl of potassium persulfate (70 mM). The mixture was stored in the darkness for 6 hours before performing the test. 100 µL of extract was added to 100 µL of $ABTS^{+}$ and the absorbance was measured at 734 nm after 15 min for 1 hour. The blank was prepared by mixing 100µL of extract with 100µL of ethanol with a control or 100 µL of methanol was mixed with 100µL of the $ABTS^{\cdot+}$ radical. The potential of the extracts to reduce the $ABTS^{\cdot+}$ radical was expressed in microgram equivalence of Trolox per milligram of dry matter (µgETx/mg DM) from the calibration line of the Trolox [11, 18, 21].

Anti-inflammation activity

Anti-inflammatory activity was assessed by determining the stabilizing potential of the membrane of red blood cells. For this, 100 µL of fresh human blood were mixed with 900 µL of sodium chloride solution (0.9%) and then centrifuged at 8000 rev/min for 10min. Then 300µL of supernatant was added to 300µL of extract and stirred for 30 min. For the negative control, 300µL of sodium chloride solution was mixed with 300 µL of supernatant. Aspirin was used as a reference compound by mixing 300 µL of aspirin (8µg/µL) and 300 µL of methanol. The samples were incubated at 56 °C for 30 min, centrifuged at 2500 rpm for 5min and the absorbance of the supernatant was measured at 560 nm. The experiment was carried out in triplicating. The percentage of stabilization of the red blood cell membrane was calculated [24-26].

$$PP= \frac{100-(A_e-A_b)}{A_{cn}}X100$$

Ae: Sample absorbance; Ab: Absorbance of white; Acn: Absorbance of negative control

Antibacterial activity of *Cordia senegalensis* leaf extracts

Evaluation of the sensitivity of bacteria to the ethanolic extract of *Cordia senegalensis.* The sensitivity test of the bacterial strains to the hydroethanolic extract of *Cordia senegalensis*, consisted of adding 100µl of bacterial inoculum to 100µl of concentrated extract at 20 mg/ml. Then the microplate was, incubated for 24 hours at 37°C. 40µl of an aqueous solution of Iodonitrotetrazolium (INT) concentrated to 0.2 mg were added to each well followed by an incubation time of 30 minutes. The appearance of a pink or red colour in as well indicates the inactivity of the extract. The tests were carried out in triplicating [27].

Determination of the minimum bactericidal inhibitory concentration

Staphylococcus aureus ATCC 6538 and *Staphylococcus epidermidis* were tested on extracts at concentrations ranging from 10mg/mL to 0.15mg/mL. The tests were carried out in triplicate. One hundred (100) µL of Mueller Hinton broth was pre-dispensed into all wells of a 96-well microplate. 100 µL of the 20 mg/mL stock solutions of extracts were added to the first and second wells. From the second wells, successive half-dilutions were made to the last wells. Then, 100 µL of concentrated bacterial broth at 10^6CFU/mL was added to all wells. After twenty (20) hours of incubation at 37°C, 40µL of an aqueous solution of iodonitrotetrazolium concentrated at 0.2 mg/mL was added to each well. The appearance of staining in the wells indicates bacterial growth. The MIC is determined by looking at the smallest concentration at which the color of the well does not turn red after addition of iodonitrotetrazolium [27].

Determination of the minimum bactericidal concentration

The minimum bactericidal concentration was achieved by inoculating on Mueller Hinton agar 10µL of the wells in which inhibition of bacterial growth was observed. After 24 hours of incubation, the smallest concentration of extract at which there is a total absence of bacterial growth on the agar corresponds to the minimum bactericidal concentration [27].

Results & Discussion

Preliminary phytochemical screening

The secondary metabolites identified in the leaves of *Cordia senegalensis* are listed in Table 1. Phytochemical analysis revealed numerous secondary metabolites in the leaves of this plant. It emerges from this table that the leaves of *Cordia senegalensis* contain flavonoids, anthocyanins, leuco-anthocyanins, mucilages, anthraquinones, sterols and terpenes. The diversity of secondary metabolites in the leaves of this plant could explain its use in the treatment of several conditions, namely skin conditions. According to the work of Perez *et al* [28], plants rich in mucilage are highly sought after for their healing properties and could be used in the production of certain preparations for local application, for the prevention of wounds and other ailments cutaneous. Likewise, the flavonoids, sterols and terpenes and anthocyanins present in the leaves of *Cordia senegalensis* are known for their antimicrobial and antiedematous activities [29-31].

Table 1: Secondary metabolites identified in *Cordia senegalensis* leaves

Secondary metabolites	
Alcaloids	-
Tannins	-
Flavonoids	+
Anthocyanes	+
Leuco anthocyanins	+
Reducing compound	-
Mucilages	+
Coumarins	-
Cyanogenic derivatives	-
Saponosids	-
Anthraquinones	+
Sterols and terpenes	+

Legend: + : present ; - : absent.

Phenolic compound content

Table 2 reports the content of phenolic compound in the ethanolic extract of the leaves of *Cordia senegalensis.* It emerges from the analysis of this table that the content of total phenols in the ethanolic extract of the leaves of *Cordia senegalensis* is (53.81±0.84) µgGAE/mgDM and that the content of total flavonoids is (12.81±0.01) µgQE/mgDM with a content of (1.463±0.375) µgCE/mgDM for condensed tannins.

Table 2: Phenolic compound content of the ethanolic extract of *Cordia senegalensis* leaves

Phenolic compound	**Total phenol content (µg GAE/mgDM)**	**Total flavonoids content (µgQE/mgDM)**	**Condensed tannin content (µgCE/mg DM)**
Ethanolic extract	53.81±0.84	12.81±0.01	1.463±0.375

Legend: µgGAE/mgDM: microgram Gallic acid equivalent per gram of dry matter; µgQE/mgDM: microgram Quercetin Equivalent per milligram of dry matter; µgCE/mgDM: microgram catechin Equivalent per milligram of dry matter.

DPPH free radical scavenging assay

The levels of trapping of the DPPH radical after 15 min, 30 min, 45 min and 60 min of reaction as a function of the concentrations of the ethanolic extract of *Cordia senegalensis* leaves are indicated in figure1. After 15 min of reaction, it is noted that the reaction is complete. For all the concentrations of this extract tested, a progressive increase in the percentage of radical trapping is noted before note a level. These curves were used to determine the concentration of the extract which causes a loss of 50% of the DPPH radical. As lower is this concentration, as more interesting is anti-free radical activity. According to the curves in Figure1, the concentration of the ethanolic extract of *Cordia senegalensis* allowing to trap 50% of the DPPH radical (IC_{50}) is 0.125µg/µL. The interesting anti-free radical activity of the ethanolic extract of *Cordia senegalensis* could be explained by its total phenol compound content. *Cordia senegalensis* ethanolic extract could be used to combat sudden free radical attack on the skin.

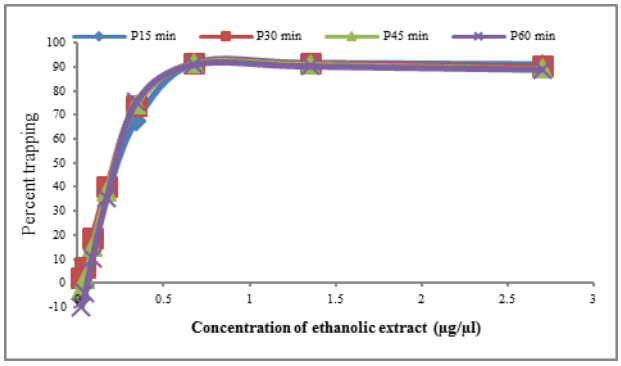

Figure 1: Percentage of DPPH radical scavenging by the ethanolic extract of Cordia senegalensis leaf

Total antioxidant capacity of the ethanolic extract of *Cordia senegalensis* leaves

The total antioxidant capacity of the ethanolic extract of *Cordia senegalensis* is expressed in microgram equivalent of ascorbic acid per milligram of dry matter (µgEAA/mgDM) using the calibration curve plotted with ascorbic acid.

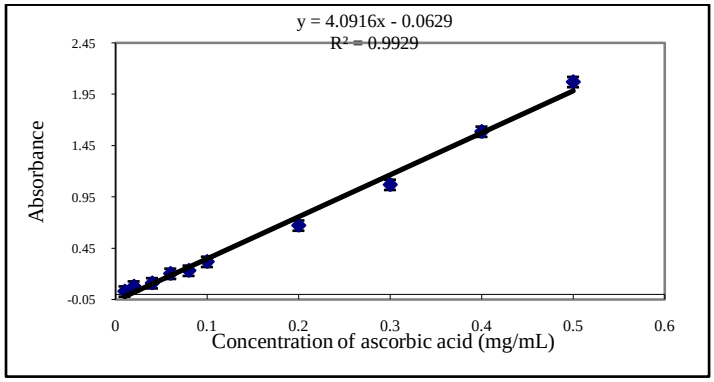

Figure 2: Calibration curve for the evaluation of the total antioxidant capacity

This total antioxidant capacity is (0.0046±0.0001)µgEAA/mgDM for the ethanolic extract of this plant. The interesting activity of this extract is linked to its content of phenolic compounds.

Potential of the extracts to reduce the $ABTS^{\cdot+}$ radical

The calibration curve for the determination of the potential of the extracts to reduce the $ABTS^{\cdot+}$ radical established with the trolox gives (y=-0.0057x+0.3783; R^2=0.9989); (y=-0.0051+0.337; R^2=0.9956); (y = -0.0045+0.3086; R^2=0.9976); (y=-0.0042+0.2872; R^2=0.998) respectively after 15min, 30min, 45min and 60 min of reaction (figure2). This potential of the ethanolic extract of *Cordia senegalensis* leaf to reduce the $ABTS^{\cdot+}$ radical cation as a function of reaction time is expressed in micrograms Equivalence of trolox per milligram of dry matter.

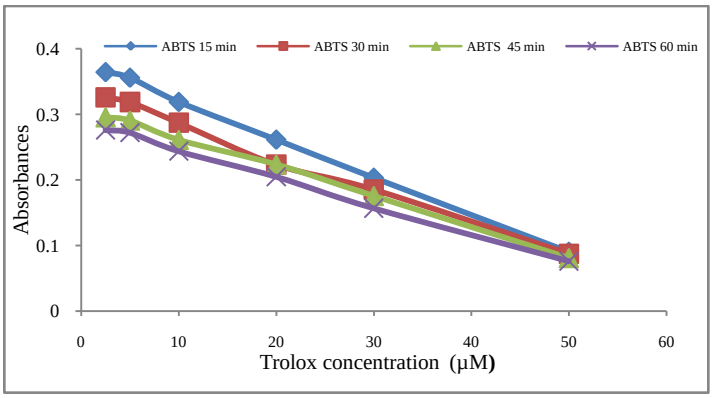

Figure 2: Calibration curve for the potential of the extracts to reduce the $ABTS^{\cdot+}$ radical

The potential of the extracts to reduce the $ABTS^{\cdot+}$ radical as a function of the reaction time expressed in micrograms Equivalence of trolox per milligram of dry matter is given in Table 3. The reduction potential of $ABTS^{\cdot+}$ varies from (103.719±7.122)µgEqTx/mgMS after 15 min of reaction at (65.414±8.768)µgEqTx/mg MS after one hour of reaction. Therefore, the potential of the ethanolic extract of *Cordia senegalensis* leaf to reduce the radical cation $ABTS^{\cdot+}$ gradually decreases over time. From the analysis of this table, it emerges that the ethanolic extract of *Cordia senegalensis* leaf showed interesting antioxidant activity. This noted activity would be due to the content of phenolic compounds in this plant. In view of the interesting antioxidant activity of the ethanolic extract *Cordia senegalensis*, it could be used to fight against free radical attacks suffered by the skin.

Table 3: Potential of the extracts to reduce the $ABTS^{\cdot+}$ radical

Reduction potential of ABTS by the ethanolic extract of *Cordia senegalensis* leaf (µgEq Tx/DM)			
15 min	30 min	45 min	60 min
103.719±7.122	87.633±8.143	77.715±9.840	65.414±8.768

Antibacterial activity of *Cordia senegalensis* leaf extracts

The Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of *Cordia senegalensis* leaf extracts are shown in Table 3.

From the analysis of this table, it emerges that the ethanolic and hydroethanolic extracts of *Cordia senegalensis* inhibited the strain of *Staphylococcus epidermidis* at a concentration of 10mg/mL. Concerning the strain of *S. aureus*, the hydroethanolic extract inhibited at a concentration of 5mg/mL while the ethanolic extract was shown to be inhibitory at a concentration of 10mg/mL against the strains of *S. aureus* and *S. epidermidis*. At tested concentrations, the ethanolic and hydroethanolic extracts of *Cordia senegalensis* are only bacteriostatic. The antibacterial activity of *Cordia senegalensis* extracts against these strains could justify its use to treat skin infections in traditional medicine.

Table 3: MIC and MBC of *Cordia senegalensis* leaf extracts

Strains	**Extract**	**Concentration (mg/mL)**	
		MIC	MBC
Staphylococcus aureus	Ethanolic	10	10 >
	Hydroethanolic	5	10 >
Staphylococcus epidermidis	Ethanolic	10	10 >
	Hydroethanolic	10	10 >

Legend: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration

Anti-inflammatory activity

The percentages of membrane stabilization of red blood cells are ethanolic extract of *Cordia senegalensis* leaf and aspirin are given in Table 4.

At a concentration of the ethanolic extract of *Cordia senegalensis* leaf of 8µg/µL, the stabilization potential of the human red blood cell membrane is (91.390±0.011)%. For an aspirin concentration also equal to 8µg/µL, it gave a stabilization percentage of (38.450±0.120)%. The analysis of this result shows that the ethanolic extract of *Cordia senegalensis* leaf showed more interesting anti-inflammatory activity than the aspirin used in this study as a reference compound. The interesting anti-inflammatory activity shown by the ethanolic extract of *Cordia senegalensis* is thought to be due to its secondary metabolite content, in particular its flavonoid content [32, 33].

Table 4: Red blood cell membrane stabilization potential

Reference extract and compound	Percentage of membrane stabilization (%)
Ethanolic extract of *Cordia senegalensis*	91.390±0.011
Aspirin	38.450±0.120

Conclusion

Plants have always been an essential source of medicine. Today, the majority of the world's population, especially in developed countries, can only be cured with traditional herbal remedies. The present study focused on phytochemical, antioxidant, antimicrobial, and antiinflammatory properties of *Cordia senegalensis.* From the results obtained, it emerges that *Cordia senegalensis* has many secondary metabolites including flavonoids, anthocyanins, leuco anthocyanins, mucilages, sterols and terpenes which can be enhanced in the fight against dermatoses. Regarding antioxidant activity, the ethanolic extract showed interesting activity. The ethanolic and hydroethanolic extracts have been shown to be bacteriostatic against strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The ethanolic extract of *Cordia senegalensis* showed more pronounced anti-inflammatory activity (P=91.390±0.011) than aspirin (P=38.450±0.120) which is a reference compound. The results of this study justify the use of *Cordia senegalensis* in traditional medicine. Ethanolic extract of *Cordia senegalensis* could be used to fight against free radical attacks on the skin, to treat skin conditions and for the preservation of perishable food products instead of using synthetic products to avoid undesirable and toxic side effects on humans being.

Acknowledgment

The authors of this manuscript would like to thank the university agency of the Francophony which funded this research project within the framework of the scholarship program "Eugen Ionescu 2019-2020"

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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