

Phytochemical Screening and Antimicrobial Activity of *Desmodium ramosissimum*

Badé Farid Toyigbénan¹, Dah-Nouvlessounon Durand¹, Sina Haziz¹, Nanoukon Chimène¹, Moussè Wassiyath¹, Nabéde Aklesso¹, Assogba Sylvestre¹, Tohoyéssou Majoie¹, Nounagnon Martial¹, Halfane Lehmane¹, Adjanohoun Adolphe², Savadogo Aly³, Baba-Moussa Lamine^{1*}

¹Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Science and Technology, University of Abomey-Calavi, Cotonou, Benin

²South Agricultural Research Centre, National Agricultural Research Institute of Benin, Attogon, Benin

³Laboratoire de Biochimie et d'Immunologie Appliquée (LABIA), UFR en Sciences de la vie et de la terre, École Doctorale Sciences et Technologies, Université Ouaga I Pr-Joseph KI-ZERBO, Ouagadougou, Burkina Faso

Email: *laminesaid@yahoo.fr, badefarid@gmail.com

How to cite this paper: Toyigbénan, B.F., Durand, D.-N., Haziz, S., Chimène, N., Wassiyath, M., Aklesso, N., Sylvestre, A., Majoie, T., Martial, N., Lehmane, H., Adolphe, A., Aly, S. and Lamine, B.-M. (2020) Phytochemical Screening and Antimicrobial Activity of *Desmodium ramosissimum*. *American Journal of Plant Sciences*, 11, 51-63.

<https://doi.org/10.4236/ajps.2020.111005>

Received: December 2, 2019

Accepted: January 10, 2020

Published: January 13, 2020

Copyright © 2020 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Microbial infections threaten the health of the Beninese population. **Objectives:** To identify bioactive phytochemical groups and evaluate the antimicrobial activity of *Desmodium ramosissimum* used in traditional medicine in Benin against microbial infections. **Methodology & Results:** The classic reactions in solution helped highlight alkaloids, anthocyanins, flavonoids, quinones, saponins, steroids, tannins, terpenoids, mucilage, Coumarin, reducing compounds, anthracene, glycosides. The antibacterial activity was evaluated by extracts dilution's method (ethanol extracts, methanol extracts and hexane extracts). The methanol extract inhibited the growth of all tested germs, with a significant effect on *E. coli* and *S. aureus* strains whose MIC values are equal to 1.25 mg/ml. On the contrary, this extract has a low activity on *P. vulgaris* strain with a MIC of 10 mg/ml. The ethanol extract also inhibited the growth of all the tested strains, but with more intense activity than the methanol extract. The strains of *C. albicans* and *S. oralis* were more sensitive to the extract with 0.625 mg/ml for MIC, while *S. aureus* and *P. vulgaris* were less sensitive (MIC = 5 mg/ml). The residual ethanol extract exerted inhibition on all microorganisms at a concentration of 20 mg/ml, except the strain of *E. faecalis* that was resistant. **Conclusion & Applications:** This study shows that besides its food virtues of this plant, there are antibacterial and antifungal properties.

Keywords

Desmodium, Benin, Extracts, Antimicrobial, Screening

1. Introduction

In developing countries such as Benin, infections are serious public health problems that threaten populations because of their frequency and severity associated with low access to health care [1].

The use of synthetic antibiotics is one of the most effective means available for humanity to face these microbial invasions. Unfortunately, today there is an increasingly growing resistance of germs against these antibiotics [2]. It is important to notice that the most affected by these infections have almost no access to primary health care. They usually use traditional medicine whose effectiveness is most of the time devoid of scientific evidence [3]. Thus, it is urgent to screen traditional medicines to assess their effectiveness and serve as the start point for the discovery of new molecules that can enrich the current armamentarium.

Desmodium ramosissimum is consumed as food but it is also used in traditional medicine against various infections including salmonellosis, candidiasis, diarrhoea, abdominal pain, wounds, diphtheria, dysentery, haemophilia, hepatitis and pneumonia [4]. This study aims to verify if beyond its food use, this plant could help support the above pathologies. It therefore proposes to evaluate the antibacterial and antifungal activity of aqueous, methanol and ethanol extracts of the leaves and stems of *Desmodium ramosissimum*. These microbiological tests covered several bacteria such as staphylococci, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Micrococcus luteus*, *Streptococcus oralis*, *Proteus vulgaris*, *Enterococcus faecalis* and, *Escherichia coli* involved in several diseases that this plant is assumed to treat. The knowledge of the chemical composition of this plant is also part of this study's objectives.

2. Material and Methods

2.1. Collection of Plant Material

The leaves and stems of *D. ramosissimum* were collected in March 2018 in the town of Porto-Novo. Once in the laboratory, the leaves and stems were washed with water then dried at a temperature of $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 16 days. They were then powdered using a Retsch mill type SM 2000/1430/Upm/Smfet. The powder obtained was used for the identification of bioactive chemical groups and for the preparation of different extracts.

2.2. Microbial Strains

The microorganisms used include both bacteria and yeast

- Ten reference strains including five Gram + bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Micrococcus luteus* ATCC 10240, *Streptococcus oralis* NCTC 8029 and *Enterococcus faecalis* ATCC 29212), four bacteria Gram- (*Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* A24974, *Proteus vulgaris* A25015, *Escherichia coli* ATCC 25922) and one yeast (*Candida albicans* MHMR).
- Four strains of *Staphylococcus* genus (*S. hominis*, *S. simulans*, *S. aureus*, *S.*

xylosus) Isolated from meat products of food origin [5].

- Three clinical strains of the multi-resistant *Staphylococcus* genus isolated from urine (*S. hemolyticus*) and blood (*S. hemolyticus* and *S. lungdunensis*) of patients at National Hospital and University Center of Cotonou [6].

All these tested strains belong to the collection of Biology and Molecular Typing Microbiology Laboratory.

2.3. Obtaining Extracts

- Methanol extract and Water-ethanol extract

The extraction method described by [7] was used. A mass of 50 g of powder of the leaf and stem was macerated in 500 ml of solvent: methanol and water ethanol mixture in the proportions 30:70 (v/v) under stirring for 48 hours. The macerated was then filtered thrice with cotton wool and once on the filter paper Whatman # 1. The extract recovered by filtration was subjected to evaporation in a rotary evaporator (RE 300). The resulting residues were dried at 40°C in an oven and the collected powders constitute the methanol extract and water-ethanol extract ready for use.

- Successive extraction with hexane, ethanol, and ethyl acetate

The used extraction technique is an adaptation of the protocol used in the work of [8] and [9]. A mass of 100 g of *Desmodium ramosissimum* powder was macerated in a liter of ethanol 96° under continued stirring for 72 hours. The obtained mixture was filtered thrice. To the filtrate, 50 ml of distilled water and 50 ml of hexane were added. The obtained mixture was then poured in a separator funnel where the two phases of the formation are observed: a lower aqueous phase and an upper organic phase. The organic layer was collected then dried to obtain the hexane extract. The first half of the filtrate of the lower layer was dried at 40°C to obtain the ethanol extract. As for the second half 200 ml of distilled water and 100 ml of ethyl acetate were added. The resulting mixture was left decanted to obtain two phases. The upper layer was recovered and dried to obtain the extract of ethyl acetate. The lower phase was dried to obtain the residual ethanol extract. The extracts obtained were stored in sterile bottles for future use.

2.4. Preparation of Standard Inoculum

The bacterial suspension in physiological water was carried out by emulsifying a few colonies of bacteria taken from the Mueller Hinton agar to achieve a turbidity equivalent to that of the standard range 0.5 McFarland. To do this, after stirring vigorously the turbidity standard on a vortex, a comparison of the turbidity of the bacterial suspension to that of the standard range 0.5 McFarland was made. Bacterial density (0.5 McFarland), was adjusted with physiological water. Each bacterial inoculum was used in 15 min to 30 min following its preparation.

2.5. Preparation of the Control

Three Controls were used. The first consisted only of the culture medium (2 ml)

to check the sterility of the workplace; the second contained the culture medium and the extract (1:1) to verify the sterility of extracts and have the reference extract-culture medium system; the third consisted of erythromycin as a reference antibacterial substance to assess the behaviour of an active substance in the medium and verify the effectiveness of the test.

2.6. Microorganisms Sensibility Tests

Seeding was carried out using a sterile cotton swab introduced into the bacterial suspension with removal of the excess. The entire agar surface was swabbed in three directions.

Sterile disks of blotting paper were firmly placed on the surface of the inoculated and dried agar. The disks placed on the boxes were carefully impregnated with 30 μ l plant extracts with a concentration of 20 mg/ml using a micro pipette. Incubations took place ideally within 15 minutes (without exceeding 30 minutes) following the deposit of disks at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

2.7. Determination of the Minimum Inhibitory Concentration (MIC)

The method of macro-dilution in liquid medium described by [10] was used for MIC determination. It's consisted in interacting germs and extracts (at various dilutions) with visual assessment of the growth of microorganisms after 24 hours of incubation [11]. The minimum inhibitory concentration is the lowest concentration at which the extract prevented visible growth of bacteria incubation from 18 to 24 hours. For its implementation, a known charge of bacterial inoculum (10^6 CFU/ml) was put in contact with a dilution range of plant extracts. The procedure is summarized as follows: In a series of 10 test tubes numbered from T1 to T10, 1 ml of sterile distilled water was introduced except in tube T1. One millilitre of the extract at the starting concentration (sensitivity test concentration) was then introduced into the tubes T1 and T2. From the tube T2, successive dilutions of 1/2 were performed up to tube T9. To all the tubes, it was added 1 ml of inoculum at 10^6 CFU/ml of MH nutrient broth. At final all the tubes contained a total volume of 2 ml and, were incubated at 37°C . After 24 h of incubation, the tubes turbidity was examined and compared to the control tube T10.

2.8. Determination of Minimal Bactericidal Concentration (MBC) or CMF

It was made in conjunction with the MIC determination. After identifying the MIC, all tubes starting from the MIC to the highest concentrations, were inoculated with a platinum loop on Petri dishes containing agar MH medium. These plates were incubated at 37°C for 24 h. Upon observation, the concentration of the extract that reveals no microbial growth is the Minimum Bactericidal Concentration (MBC). Microbial growth was visually checked. Consistent with [12]

and [13], the MBC is the lowest concentration at which the extract has prevented visible bacterial growth after transplanting and the CMF, the lowest concentration at which the extract prevented visible growth of fungi after transplanting.

2.9. Effect of the Extracts and Categorization

The effect of the extracts was determined by taking the ratio CMB (or CMF)/MIC. If the ratio is less than or equal to 4, the extract is said bactericidal (or fungicidal); if, on the other hand, it is greater than 4 the extract is bacteriostatic or fungi static as appropriate [14].

2.10. Preliminary Chemical Screening

The chemical screening used the standard reactions in solution based on colouration, precipitation or the formation of foam. It consisted in finding bioactive groups with antimicrobial potential notably alkaloids, anthocyanins, flavonoids, quinones, saponins, steroids, tannins, terpenoids, mucilage, coumarins, reducing compounds, anthracene, and glycosides. Furthermore, the research of cyanogenic glycosides will give information about possible toxicity to cyanide. Alkaloids were detected by using the reagents of Dragendorff according to the method described by Hager and Wagner [15]. Flavonoids have been identified with the reagent of Shinoda when the mixture of aqueous acid extract and magnesium shavings revealed a pink-red or red-purple colouration; as for anthocyanins, their presence is revealed by a red colouration in the absence of magnesium shavings in the above mixture [16]. Cyanogenic glycosides have been underlined by the reaction of picric acid with an aqueous decoction's vapour when picric acid paper changed to orange or red [17]; Quinones were found by reacting ether extracts with 1% KOH. The appearance of a characteristic colour ranging from orange-red to violet-purple indicated their presence; Saponins were detected by their ability to form a foam (height: >10 mm) after the stirring of an aqueous solution [18]. Steroids have been researched by the reaction of Liebermann-Burchard. It consisted in putting the ethereal organic extract in the presence of anhydrous acetic acid and concentrated sulphuric acid and observe a purple or green colouring; Tannins have been investigated by reacting an aqueous infused with a ferric chloride solution at 1%. The reaction was considered positive with the formation of blue-green precipitates, dark blue or green [18]; the Mixing of the aqueous infused with Stiasny reagent helped found catechol tannins while adding sodium acetate and ferric chloride to the filtrate resulting in the formation of precipitates, helped found Gallic tannins [19]. Tannins have been investigated by reacting an aqueous infused with a ferric chloride solution at 1%. The reaction was considered positive with the formation of blue-green precipitates, dark blue or green [18]; Mixing the brewed with the reagent Stiasny helped found catechol tannins while adding sodium acetate filtrate and ferric chloride resulting in the formation of precipitates, helped found Gallic tannins [19].

2.11. Statistical Analysis

The standard errors of means of experiments were analyzed and analysis of data was performed using GraphPad Prism 8. Differences were taken as statistically significant when $p < 0.05$.

3. Results and Discussion

Extraction yield

The yields of the various extractions are summarized in **Table 1**. They are expressed as a percentage relative to the mass of the initial powder. The analysis of this table shows that the extraction yields vary according to the extract type. And that the best yield is that of the ethanol extract (16%) while the hexane extract has the lowest yield (2.24%).

Sensitivity of reference's strains to different extract

The inhibition zones' results of the ten (10) reference strains over time (24 h and 48 h), are recorded on the **Figures 1-3**. The analysis of these figures reveals that the larger zones of inhibition of methanol extracts, ethanol and residual ethanol extracts were observed respectively on *Micrococcus luteus* strains (14.5 ± 0.6 mm), *E. coli* (18.6 ± 0.02 mm) and *Streptococcus oralis* (15.6 ± 0.02 mm). It should also be noted that the lowest measured inhibition diameter is that of *Staphylococcus epidermidis*' strains (9 ± 0.5 mm), and therefore considered to be the least sensitive microorganism. Analysis of variance shows that there is no difference ($p > 0.05$) over time (between 24 and 48) between the inhibition's diameters per strains. However, it shows a very highly significant difference ($p < 0.001$) between treatments. Furthermore, no zone of inhibition was observed around the disks impregnated with water-ethanol extracts, hexane and ethyl acetate extracts.

Sensitivity of Foodborne Strains

Figure 4 shows the inhibitions' diameters of ethanol extracts, acetate, water-ethanol, methanol and hexane extract of *D. ramosissimum* on the in vitro growth of foodborne strains. The analysis of this figure shows that the largest diameters of inhibition were observed with the ethanol extract on the *S. xylosus* strain (13.5 ± 0.07 mm), followed by hydro-ethanol extract on *S. hominis* strain (13.2 ± 0.8 mm). No significant differences ($p > 0.05$) were observed in the inhibition's diameter after 48 hours.

Table 1. Yield of extraction.

Extract	Mass of powder (g)	Mass of extract (g)	Yield (%)
Ethanol	50	8	16
Water-ethanol	50	6.7	13.4
Methanol	50	7.39	14.78
Ethyl acetate	50	2.6	5.2
Hexane	50	1.12	2.24
Residual ethanol	50	2.11	4.22

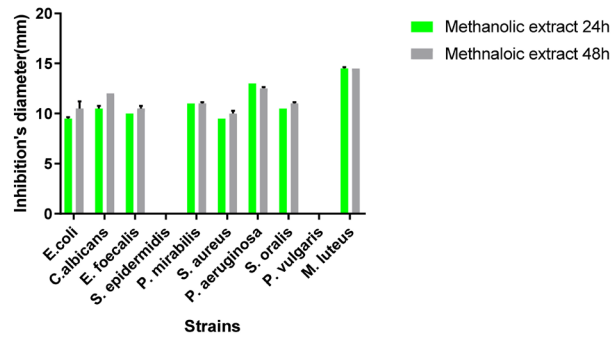


Figure 1. Inhibitions' diameters of the methanol extract on reference strains as over time (24 h and 48 h).

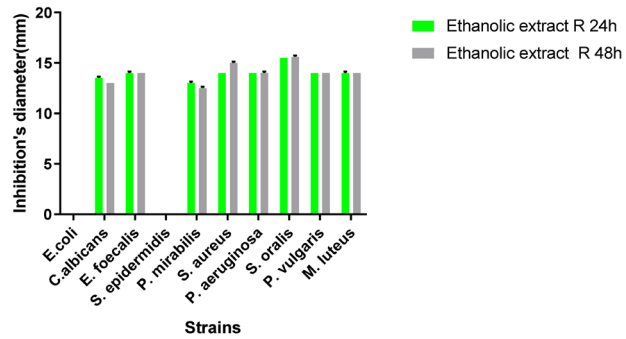


Figure 2. Inhibitions' diameters of residual ethanol extract on reference strains over time (24 h and 48 h).

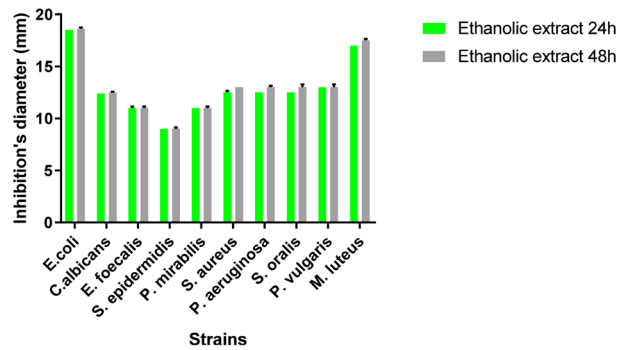


Figure 3. Inhibitions' diameters of ethanol extract on the reference strain over time (24 h and 48 h).

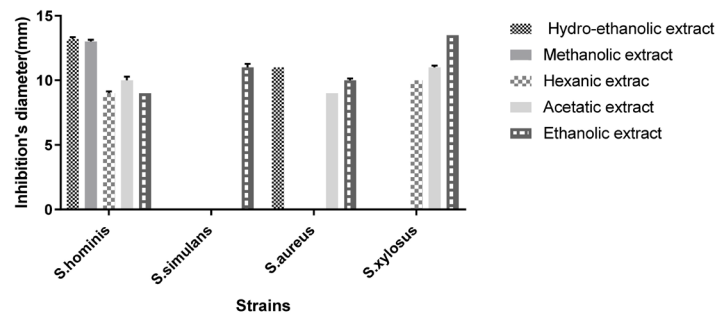


Figure 4. Inhibition diameters of ethanol, acetate, water-ethanol, methanol and hexane of *D. ramosissimum* on the in vitro growth of food's strains.

Sensitivity of Multi-Resistant Clinical Strains of Staphylococcus

The sensitivity of clinical strains of Staphylococcus to our extract is shown in Figure 5. We note that the largest diameter of inhibition (20 ± 0.08 mm) was observed on *Staphylococcus lungdunensis* strain with the methanol extract. Apart from methanol and ethanol residual extracts residual that inhibited each 33.33% (1/3) of strains, hexane, acetate and water-ethanol extracts showed no inhibitory effect on multi-resistant strains of Staphylococcus.

Minimum Inhibitory and Bactericidal Concentrations of *D. ramosissimum* extract on the different reference strains used

From the analysis of results relating to MIC (Tables 2-4), the methanol extract inhibited the growth of all tested germs, with a significant effect on *E. coli* and *S. aureus* strains where its MIC values are equal to 1.25 mg/ml. However, this extract has a low activity on *P. vulgaris* strain with an MIC of 10 mg/ml.

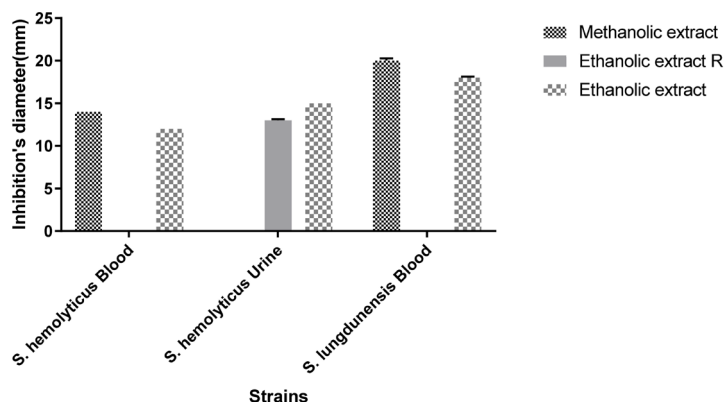


Figure 5. Inhibition diameters of ethanol, residual ethanol and methanol extracts of *D. ramosissimum* on in vitro growth of clinical strains.

Table 2. Minimum Inhibitory Concentration (MIC) and bactericidal (MBC) in mg/ml of *D. ramosissimum*'s methanol extracts and MBC/MIC ratio.

Strains	methanol extract		
	MIC (mg/ml)	MBC (mg/ml)	MBC/CMI
<i>Etherococcus foecalis</i>	2.5	10	4
<i>Streptococcus oralis</i>	2.5	10	4
Gram+ <i>Staphylococcus aureus</i>	1.25	-	
<i>Micrococcus luteus</i>	5	20	4
<i>Staphylococcus epidermidis</i>	5	20	4
Gram- <i>Escherichia coli</i>	1.25	5	4
<i>Proteus mirabilis</i>	2.5	5	2
<i>Pseudomonas aeruginosa</i>	2.5	-	
<i>Proteus vulgaris</i>	10	-	
yeast <i>Candida albicans</i>	2.5	10	4

Table 3. Minimum Inhibitory Concentration (MIC) and bactericidal (MBC) in mg/ml of *D. ramosissimum*'s ethanol extracts and MBC/MIC ratio.

	Strains	ethanol extract		
		MIC (mg/ml)	MBC (mg/ml)	MBC/CMI
Gram+	<i>Etherococcus foecalis</i>	2.5	10	4
	<i>Streptococcus oralis</i>	0.625	10	16
	<i>Staphylococcus aureus</i>	5	-	
	<i>Micrococcus luteus</i>	1.25	10	8
	<i>Staphylococcus epidermidis</i>	1.25	5	4
Gram-	<i>Escherichia coli</i>	1.25	5	4
	<i>Proteus mirabilis</i>	2.5	10	4
	<i>Pseudomonas aeruginosa</i>	2.5	10	4
	<i>Proteus vulgaris</i>	5	-	
yeast	<i>Candida albicans</i>	0.625	10	16

Table 4. Minimum Inhibitory Concentration (MIC) and bactericidal (MBC) in mg/ml of *D. ramosissimum*'s ethanol residual extracts and MBC/MIC ratio.

	Strains	Ethanol residual extract		
		MIC (mg/ml)	MBC (mg/ml)	MBC/CMI
Gram+	<i>Etherococcus foecalis</i>	-	-	
	<i>Streptococcus oralis</i>	1.25	20	16
	<i>Staphylococcus aureus</i>	0.313	5	16
	<i>Micrococcus luteus</i>	1.25	10	8
	<i>Staphylococcus epidermidis</i>	2.5	20	4
Gram-	<i>Escherichia coli</i>	2.5	10	8
	<i>Proteus mirabilis</i>	0.625	10	16
	<i>Pseudomonas aeruginosa</i>	1.25	10	8
	<i>Proteus vulgaris</i>	2.5	-	
yeast	<i>Candida albicans</i>	0.625	10	16

The ethanol extract also inhibited the growth of all the tested strains, but with a more intense activity than the methanol extract. *C. albicans* and *S. oralis* were more sensitive to this extract with an MIC of 0.625 mg/ml, while *S. aureus* and *P. vulgaris* were less sensitive (MIC = 5 mg/ml).

The residual ethanol extract exerted inhibition on all microorganisms at a concentration of 20 mg/ml except for the *E. foecalis* strain that was resistant to this extract.

Analysis of results for the Minimum Bactericidal Concentrations varies depending on the strain and types of extracts. The most remarkable CMB of methanol, residual ethanol and ethanol extracts are 5 mg/ml (*E. coli* and *P. mirabi-*

lus), 5 mg/ml (*S. epidermidis*, *E. coli*) and 5 mg/ml (*S. aureus*) respectively. Furthermore, these methanol, ethanol and residual ethanol extracts did not have bactericidal effect on some strains. This is the case of *S. aureus*, *P. aeruginosa* and *P. vulgaris* for the methanol extract; *S. aureus* and *P. vulgaris* for ethanol extract and finally *E. coli* and *P. vulgaris* strains for the residual ethanol extract.

Preliminary chemical screening:

The preliminary phytochemical screening (**Table 5**) performed on the *D. ramosissimum* reveals the presence of several pharmacological groups with penitential interest. All parts of this plant contain saponins, flavonoids, anthocyanins and, tannins. It was reported that this plant is used in traditional Beninese medicine in form of decoction and since glycosides are absent, this implies that there is no risk of cyanide poisoning due to the way traditional recipes are prepared. During this study, the phenolic compounds and derivatives, like anthocyanins, flavonoids and quinones identified in *D. ramosissimum* could justify its strong antimicrobial activity observed on the studied strains according to Ebana *et al.*, 1991 [20] and Nguyi, 1988 [21]. This confirms the use of *Desmodium ramosissimum* in the wounds and ulcers' treatment (Bade *et al.*, 2018) [4].

These results could help to understand its use in traditional medicine against respiratory and epidermal diseases [4]. The literature does not seem to present in a singular way the composition of this plant; nevertheless, a study carried out by Muanda in 2010 [22] on a similar plant *D. adscendens* revealed the presence of flavonoids, steroids, tannins and saponins to which biological properties are attributed. These include, the antimicrobial activity on *Trichophyton rubrum* and *Candida albicans* due to phenols and saponins according to Nwachukwu *et al.*, 2008 [23] as well as in vivo antidiarrheal activity attributed to flavonoids, tannins and terpenoids (Sule *et al.*, 2001) [24]. It should be noted that this study confirms those earlier findings, by identifying flavonoids, steroids and saponins and shows the antimicrobial activity on *C. albicans* of this plant. In the flowers of *Cucurbita maxima*, Attarde *et al.*, 2010 [25] reported the presence of steroids from which spinastrol was isolated by Consolacio & Kathlun in 2005 [26] as well as flavonoids, saponins and other phenols (Saha & Haldar, 2011) [27]. These compounds are responsible for several properties of the flower including the antimicrobial activity on *Salmonella thyphi*, *E. coli*, *Escherichia faecalis*, *Bacillus cereus*, *Candida albicans* and *Candida lunata* (Muruganatham *et al.*, 2016) [28]. The activity on *Candida albicans* was confirmed by this study and its antibacterial spectrum was extended notably to multi-resistant *Staphylococcus*'s stains used in this study. The results obtained in this study are therefore for this species the first scientific report. Indeed, the present study reports the presence of alkaloids, anthocyanins, flavonoids, tannins, saponins and reducing compounds of this plant. Besides, the presence of quinones and terpenoids was noted in *D. ramosissimum*. From antimicrobial's activity point of view, this plant is very active.

Table 5. Qualitative phytochemical screening of the *D. ramosissimum* powder.

Secondary metabolites	Results
<i>Alkaloids</i>	++
<i>Tannins</i>	+++
<i>Flavonoid</i>	+++
<i>Anthocyanins</i>	+++
<i>Quinone Derivatives</i>	+++
<i>Saponosides</i>	+++
<i>Coumarins</i>	+++
<i>Mucilage</i>	+++
<i>Reducing Compound</i>	+++
<i>O Glycosides</i>	---
<i>Anthracene</i>	+++
<i>Terpenoids</i>	+++

4. Conclusion

This study shows that some plants consumed as food in Benin, like the *D. ramosissimum* are also endowed with great antibacterial and antifungal activities. It suggests that this aspect of the plant should be sufficiently exploited and that a thorough phytochemical screening and toxicology studies should be carried out to characterize the antimicrobial compounds contained in this plant and determine their limits of use.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Traoré, Y., Ouattara, K., Yéo, D., Doumbia, I. and Coulibaly, A. (2012) Recherche des activités antifongique et antibactérienne des feuilles d'*Annona senegalensis* Pers. (Annonaceae). *Journal of Applied Biosciences*, **58**, 4234-4242.
- [2] N'tcha, C., Sina, H., Peter, A., Kayodé, P., Gbenou, J.D. and BabaMoussa, L. (2017) Antimicrobial Activity and Chemical Composition of (Kpete-Kpete): A Starter of Benign Traditional Beer Tchoukoutou. *BioMed Research International*, **2017**, Article ID: 6582038. <https://doi.org/10.1155/2017/6582038>
- [3] Balakrishna, M., Khaki, S.S., Karuna, M.S.S., Sarada, S., Kumar, C.G. and Prasad, R.B. (2017) Synthesis and *in Vitro* Antioxidant and Antimicrobial Studies of Novel Structured Phosphatidylcholines with Phenolic Acids. *Food Chemistry*, **221**, 664-672. <https://doi.org/10.1016/j.foodchem.2016.11.121>
- [4] Toyigbénan, B.F., Raphiou, M., Marcellin, A., Durand, D.-N., Aklesso, N., Sylvestre, A., Haziz, S., Adolphe, A., Aly, S. and Lamine, B.-M. (2018) Ethnobotanical Survey of Three Species of *Desmodium* genus (*Desmodium ramosissimum*, *Desmodium gangeticum* and *Desmodium adscendens*) Used in Traditional Medicine, Benin. *In-*

- ternational Journal of Sciences*, **7**, 26-29. <https://doi.org/10.18483/ijSci.1860>
- [5] Attien, P., Sina, H., Moussaoui, W., *et al.* (2013) Prevalence and Antibiotic Resistance of *Staphylococcus* Strains Isolated from Meat Products Sold in Abidjan Streets (Ivory Coast). *African Journal of Microbiology Research*, **7**, 3285-3293. <https://doi.org/10.5897/AJMR2013.5688>
- [6] Nanoukon, C., Argémi, X., Sogbo, F., Orekan, J, Keller, D., Affolabi, D., Schramm, F., Riegel, P., Baba-Moussa, L. and Prévost, P. (2017) Pathogenic Features of Clinically Significant Coagulase-Negative Staphylococci in Hospital and Community Infections in Benin. *International Journal of Medical Microbiology*, **307**, 75-82. <https://doi.org/10.1016/j.ijmm.2016.11.001>
- [7] Dah-Nouvlessounon, D., Adoukonou-Sagbadja, H., Diarrassouba, N., Sina, H., Adjonohoun, A., Inoussa, M. and Baba-Moussa, L. (2015) Phytochemical Analysis and Biological Activities of *Cola nitida* Bark. *Biochemistry Research International*, **2015**, Article ID: 493879. <https://doi.org/10.1155/2015/493879>
- [8] Sanogo, S., Sanogo, A.K. and Yossi, H. (2006) Collecte et conservation durable des graines et d'échantillons de plantes. Rapport de recherche de la campagne 2005/2006. 12^e session de la Commission Scientifique de l'Institut d'Économie Rurale (IER), Bamako.
- [9] N'Guessan, K., Kadja, B., Zirihi, G.N., Traoré, D. and Aké-Assi, L. (2009) Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Kroubo (Agboville, Côte-d'Ivoire). *Sciences & Nature*, **6**, 1-15. <https://doi.org/10.4314/scinat.v6i1.48575>
- [10] Delarras, C. (1998) Microbiologie. 90 heures de travaux pratiques. Gaétan Morien Editeur. 169178.
- [11] Balouiri, M., Sadiki, M. and iIbnsouda, S.K. (2016) Methods for *in Vitro* Evaluating Antimicrobial Activity: A Review. *Journal of Pharmaceutical Analysis*, **6**, 71-79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- [12] Hoşgor, L.M., Ermertcan, S., Eraç, B. and Taşlı, H. (2011) An Investigation of the Antimicrobial Impact of Drug Combinations against *Mycobacterium tuberculosis* Strains. *Turkish Journal of Medical Sciences*, **41**, 719-724.
- [13] Kaya, O., Akçam, F. and Yaylı, G. (2012) Investigation of the *in Vitro* Activities of Various Antibiotics against *Brucella melitensis* Strains. *Turkish Journal of Medical Sciences*, **42**, 145-148.
- [14] Ouattara, L.H., Kabran, G.R.M., Guessennd, N.K., Konan, K.F., Mamyrbekova Bekro, J.A. and Bekro, Y.A. (2016) Antibacterial Activities *in Vitro* Extract from Root Bark of *Mezoneuron benthamianum* and Rods of *Paullinia pinnata* 2 Plants of the Pharmacopoeia Ivorian. *Revue CAMES—Série Pharmacie Médecine Traditionnelle Africaine*, **18**, 31-40.
- [15] Jayashree, D. (2013) Phytochemicals and TLC Fingerprinting Analysis of Methanol Extracts of Three Medicinal Plants. *International Journal of Pharmacy Research*, **4**, 123-126. <https://doi.org/10.7897/2230-8407.04627>
- [16] Jaradat, N.F. and Al Hussen Ali, A. (2015) Preliminary Phytochemical Screening, Quantitative Estimate of Total Flavonoids, Total Phenols and Antioxidant Activity of *Ephedra alata* Decne. *Journal of Materials and Environmental Science*, **6**, 1771-1778.
- [17] Dohou, N., Yamni, K., Tahrouch, S., Hassani, L.M.I., Badoc, A. and Gmira, N. (2003) Screening of a Phytochemical Endemic Ibero-Moroccan Thymelaea Lythroides. *Bulletin de la Société de Pharmacie*, **142**, 61-78.
- [18] Longanga, O., Vercruysse, A. and Foriers, A. (2000) Contribution to the Ethno Bo-

- tanical, Phytochemical and Pharmacological Studies of Traditionally Used Medicinal Plants in the Treatment of Dysentery and Diarrhoea in Lomela Area, Democratic Republic of Congo (DRC). *Journal of Ethnopharmacology*, **71**, 411-423.
[https://doi.org/10.1016/S0378-8741\(00\)00167-7](https://doi.org/10.1016/S0378-8741(00)00167-7)
- [19] Mustapha, B.A., Kubmarawa, D., Shagal, M.H. and Ardo, B.P. (2016) Preliminary Phytochemical Screening of Medicinal Plants Found in the Vicinity of Quarry Site in Demsa, Adamawa State, Nigeria. *American Chemical Science Journal*, **11**, 1-7.
<https://doi.org/10.9734/ACSJ/2016/21519>
- [20] Ebana, R.U.B., Madunagu, B.E., Ekpe, E.D. and Otung, I.N. (1991) Microbiological Exploitation of Cardiac Glycoside and Alkaloids from *Garcinia kola*, *Borreria ocy-moides*, *Kola nitida* and *Citrus aurantifolia*. *Journal of Applied Biotechnology*, **71**, 398-401. <https://doi.org/10.1111/j.1365-2672.1991.tb03807.x>
- [21] Nguyi, A. (1988) Tannins of Some Nigerian Flora. *Journal of Biotechnology*, **6**, 221-226.
- [22] Nsemi, M.F. (2010) Identification of Polyphenols, Evaluation of Their Antioxidant Activity and Study of Their Biological Properties. Doctoral Thesis, 295 p.
- [23] Nwachukwu, I.N., Allison, L.N., Chinakwe, E.C. and Nwadiaro, P. (2008) Studies on the Effects *Cymbopogon citratus*, *Ceiba pentandra* and *Loranthus bengwelensis* Extracts One Species of Dermatophytes. *The Journal of American Science*, **4**, 58-67.
- [24] Sule, M.I., Njinga, N.S., Musa, A.M., Magaji, M.G. and Abdullah (2001) Antidiarrheal and Phytochemical Studies of the Stem Bark of *Ceiba pentandra* (Bombacaceae). *Nigerian Newspaper of Pharmaceutical Sciences*, **8**, 143-148.
- [25] Attarde, D.L., Kadu, S.S., Chaudhari, B.J., Kale, S.S. and Bhamber, R.S. (2010) *In Vitro* Antioxidant Activity of Pericarp of *Cucurbita maxima* Duch. ex Lam. *International Journal of PharmTech Research*, **2**, 1533-1538.
- [26] Consolacio, Y.R. and Kathlun, L. (2005) Sterols from *Cucurbita maxima*. *Philippine Journal of Science*, **134**, 83-87.
- [27] Saha, P., Mazumder, U.K. and Haldar, P.K. (2011) *In Vitro* Antioxidant Activity of *Cucurbita maxima* Aerial Parts. *Free Radicals and Antioxidants*, **1**, 42-48.
<https://doi.org/10.5530/ax.2011.1.8>
- [28] Muruganatham, N., Solomon, S. and Senthamilselvi, M.M. (2016) Antimicrobial Activity of *Cucurbita maxima* Flowers (Pumpkin). *Journal of Pharmacognosy and Phytochemistry*, **5**, 15-18.