ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Phytochemical screening, antibacterial and anti-radical activities of *Daniellia oliveri* trunk bark extracts used in veterinary medicine against gastrointestinal diseases in Benin.

Koudoro Yaya Alain[1], Wotto Dieudonné Valentin [2], Konfo T. R. Christian[1], Agbangnan D. C. Pascal[1,] Sohounhloue C. K. Dominique[1*]

1. Laboratory of Study and Research in Applied Chemistry / Polytechnic School of Abomey-Calavi / University of Abomey-Calavi, 01 BP 2009 Cotonou, Benin.

2. Physical Chemistry Laboratory, Faculty of Science and Technology

Manuscript Info

Manuscript History:

Received: 18 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

Daniellia oliveri, secondary metabolites, DPPH, bactericidal, GC / MS

Corresponding Author

yayakoudoro@yahoo.fr

Abstract

In Benin, pastoral activities contribute to food security. But the herds are often victims of gastrointestinal infections that decimated livestock and causing economic losses in this sector. The majority of farmers have recourse to herbal remedies to solve many animal health problems. This work aims to study the chemical composition and biological properties of *Daniellia oliveri*, plant revealed after ethnobotanical and ethnopharmacological surveys as a plant used by farmers to treat gastrointestinal diseases of their livestock. Results showed the presence of several secondary metabolites (alkaloids, leuco-anthocyanins, reducing compounds, saponin, tannins, mucilages, flavonoids, phenols, sterols and terpenes). Phenolic compounds contents vary according to the nature of the extraction solvent. However, the crude extracts showed lower anti-radical activity compared to ethyl acetate fractions (IC_{50} = 0.25µg / ml) and ethyl ether (IC_{50} = 0.06 µg / ml). The latter are more active than the synthetic compounds, BHA (IC_{50} = 4.8 µg / ml), quercetin (IC_{50} = 3 µg / ml) and gallic acid (IC_{50} = 0.9 µg / ml). The ethanol and hydroethanolic extracts of this plant showed bactericidal activity against *Escherichia coliand Klebsiellapneumoniae*. Twenty-two molecules were characterized from hydroethanolicextract of *D. oliveri* whose ethanoate glycerol, catechol and octadeca-9-enamide as major coumpound.

INTRODUCTION

Agriculture in the world provides a means to more people than any other industry. Agricultural production and productivity must be increased to raise rural incomes and support the growing number of people dependent on the industry and to provide food and raw materials needed by increasing urban populations (Faye, 2001). According to Ziegler, (2005), nearly one billion people are food insecure; 36 million people died due to nutrient deficiency and every second one person dies of hunger in the world. Livestock farming is an important source of livelihood for about one billion people, including 800 million poor people. It affects natural resources, public health, social equity and economic growth. With a world population expected to reach nine (09) billion people by 2050, livestock will play a vital role in fighting against hunger. Whereas world livestock products have more than kept pace with human population, the situation is not the same in all regions. Production levels have increased rapidly in Asia, Latin America and the Caribbean. However, growth in sub-Saharan Africa, where livestock is among the first activities undertaken by man, behind agriculture, to ensure food security was very slow (Tamboura *et al*, (1998). At this low productivity must be added the difficulties related to gastrointestinal diseases that are the major causes of mortality of livestock and problems due to free radicals that reduce drastically the vitality of these animals, their growth and their resistance to

pathologies (Anderson *et al*., 1991). Animal diseases cause significant economic losses for both breeders as regional and national levels. Unfortunately, the health control of animals has repercussions for the consumer and the environment. Chemicals (antibiotics) and pesticides (against parasites and disease transmission) may have direct effects on the environment by affecting non-target organisms or animals, thus disturbing the ecosystem functioning (FAO, 1984). In addition, the imprudent and excessive use of animal drugs, mainly antibiotics, can lead to the selection of resistant pathogens, causing a continual search of new molecules and modification of microbial ecology (Uilenberg 1998). However, the last ten years, herbal veterinary medicine has developed considerably, both for pets and for farm animals. Thus, several laboratories commercialize herbal medicines for both acute and chronic diseases (Arnaud, 2009). Baerts and Lehmann (1991) developed an inventory of 495 veterinary medicinal plants in Burundi. These same authors (Baerts, Lehmann, 1993) presented a lexicon of 34 names in Kirundi related to livestock diseases. The veterinary art and traditional medicines were the main preoccupations of Ba (1994) whose workshave focused on theFulani, while a collective work (Anon., 1996), directed by a team of 40 people (veterinarians , agronomists, farmers, traditional healers, etc.), was investigated in Kenya sixty livestock diseases. More recently, Byavu et al. (2000) inventoried plants used for the treatment of cattle on farms in the Ruzizi plain in Congo. The ethnobotanical and ethnopharmacological surveys conducted jointly by botanists and veterinarians throughout the territory of Benin have shown that *D. oliveri* is part of the plants used by farmers to treat gastrointestinal diseases of livestock whose chemical and biological potential remains unknown (Dassou*et al*., 2014;. Ogni *et al*., 2014). In order to correct this scientific insufficiency the present work aims valuing *D.oliveri* acclimated in Benin through its phytochemical screening and evaluation of its antibacterial and anti-radical activities. This could justify its use in veterinary pharmacopoeia.

MATERIAL AND METHODS

Material

The plant materialused in thisstudyconsists of the trunkbark of *Daniella oliveri*collected in Bembérékè (Latitude/longitude: 10°13'44" N/2°39'55" E), sud areas of Benin.*Escherichia coli* (O: 157H7) and *Klebsiella pneumoniae* (ATCC35657) provided by the National Health Laboratory of Beninwere the strainstested in this study.

Methods

After ethnopharmacological and ethnobotanical surveys conducted jointly by botanists and veterinarians throughout the Benin territory, the trunk bark of *D. oliveri* was harvested and dried at laboratory temperature (25°C30°C) until its stabilization and reduction in powder.

Identification of secondary metabolites

Secondary metabolites were determinated by staining and precipitation reactions specific to each metabolite family:

Flavonoids: Flavonoids identification was carried out by the test of cyanidin (Bruneton, 1999).

Tannins: They have been highlighted by the Stiasny test (Soro*et al*., 2009).

Saponins: The saponins were determined by foam test; degree of aqueous decoction dilution giving persistent foam after shaking (Dohou *et al*., 2003).

Phenols: Identification of compounds belonging to the group of phenols was made by the reaction with ferric chloride (Dohou *et al*., 2003).

Terpenes and sterols: Sterols and terpens have been identified by the Liebermann-Burchard test (Békro*et al*., 2007).

Alkaloids: Alkaloids were identified by Meyer test and confirmed by Bouchardat test (N'guessan*et al*., 2009).

Anthraquinone: They were identified by Bornträger test (Bruneton, 1999).
Mucilages: Their presence has been detected by introducing an aqueous decoction in ethyl ether. Obtaining a decoction of a flocculent precipitate in ethyl ether indicated the presence of mucilages (Traore, 2010).

Coumarins: Coumarins were identified by UV fluorescence at 365 nm (Soro*et al.*, 2009).
Volatile compounds: The volatile compounds were identified by the hydro distillation method using an extractor of Clevenger type (Clevenger, 1928; Alitonou*et al*., 2012).

Preparation of extracts

The technique used was that of maceration. 50g of each powder sample were introduced into a 500 ml flask containing 250 ml of extraction solvent (ethanol, water or ethanolwater 50/50). The flask was stoppered and stirred continuously for 72 hours. After filtration, the extracts were evaporated to dryness at 40 °C using a rotary evaporator (Heidolph kind). Differnents fractions were obtained by successive partitions with solvents of increasing polarity: hexane, dichloromethane, ethyl ether, ethyl acetate and butane (figure1). Extraction yield (Y) was calculated by the formula below:

$$y = \frac{\text{Mass of extract}}{(\text{Mass of plant material used})} X100$$

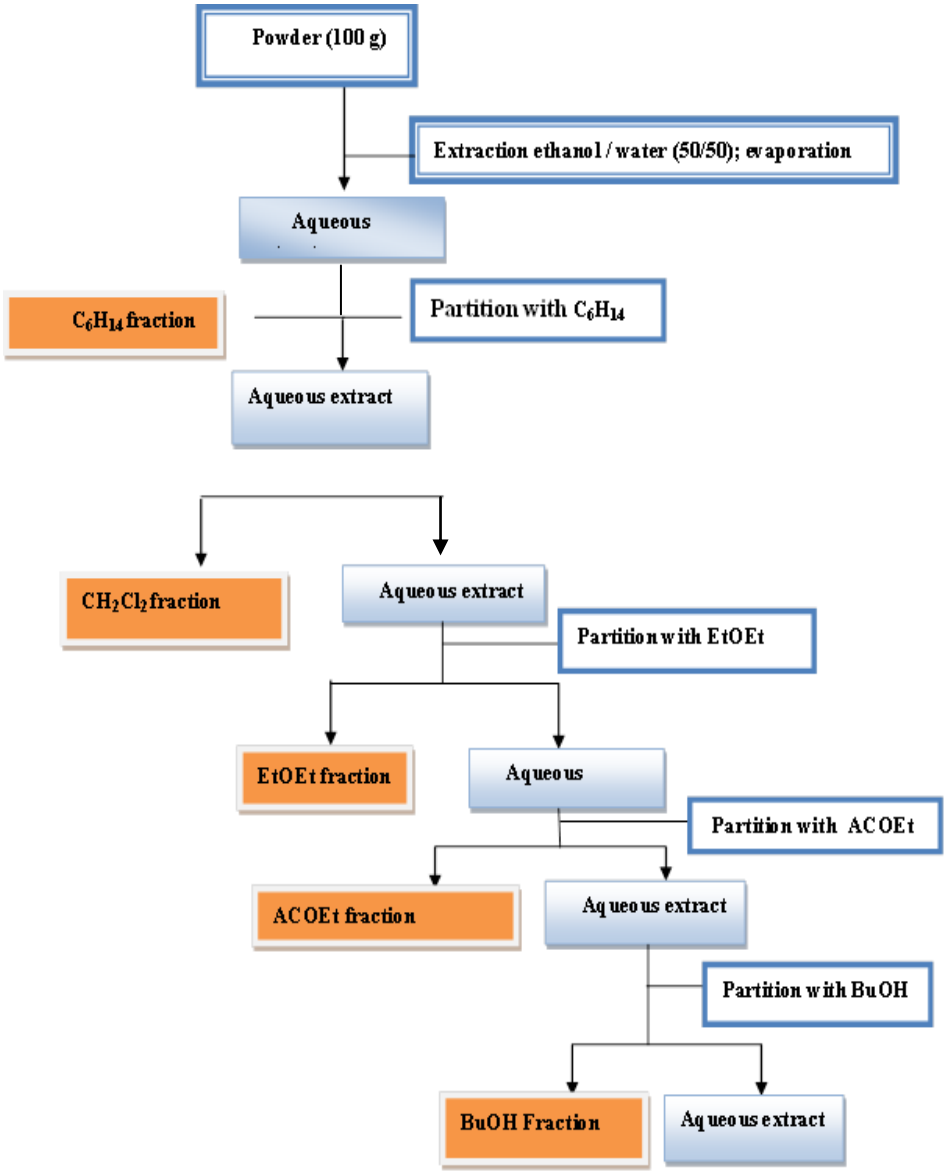


Figure 1. Extraction diagram with increasing polarity solvents.

Determination of phenolic compounds

Total polyphenols: The total phenolic content of the various extracts was quantified using the Folin–Ciocalteu method (Singleton *et al*., 1999; Siddhuraju *et al*., 2007). This method consists to use a mixture of phosphotungstic and phosphomolybdic acids which was reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum (Ribéreau-Gayon, 1968). The absorbance was measured by a spectrophotometer (JENWAY 50/60 Hz) at 765 nm. Gallic acid was used as reference and the total phenol content in the extract was expressed by mg of Gallic acid equivalent per gram of dry matter.

Total Flavonoids: The method of aluminum trichloride ($AlCl_3$) was used to quantify the total flavonoids. This technic was based on the formation of the aluminum-flavonoids complex which had a maximum absorption at 500 nm (Bahorun *et al*., 1996; Agbangnan *et al*., 2012)

Condensed tannins: The condensed tannins dosing was achieved by the method of sulfuric vanillin (Schofield *et al*., 2001; Xu *et al*., 2007). The principle of this assay was based on the binding of vanillin aldehyd group on the carbon in position 6 of the ring of the catechol to form a redcolored complex chromophore which absorbed at 510 nm.

Evaluation of scavengingactivity

The scavenging activity was evaluated by the DPPH method. The principle of this method was based on measuring the trapping free radicals in a solution of DPPH. This trapping was indicated by the disappearance of the purplecolor of DPPH. The mixture of DPPH solution and the sample was left in the darkness for one hour and the absorbance measured at 517 nm Brand-Willams *et al*., 1995; Agbangnan *et al*.,2013). The trapping percentage was determined by the formula: $P = \frac{AbW - AbS}{AbW}$X100 P: percentage of trapping; AbW: absorbance of the white; AbS: Absorbance of the sample

Determination of antibacterialactivity

The antibacterial activity was evaluated in microplates and in Petri dishes according to literature (NCCLS, 2003; Yehouenou *et al*., 2010).

GC-MS analysis of the extract

D. oliveri composition was analyzed by GC-MS using a Varian Gas Chromatograph CP3800 coupled with Mass Detector 1200 MS. The splitless injector model 1177 was at 280°C. The CP 8400 Auto sampler was injecting each time 1 µL of extract sample. The carrier gas was helium, at a working constant flow rate of 1 mL/min. GC-MS analysis was carried out using a fused silica capillary column Factor Four VF-5 ms, measuring 30 m x 0.25 mm internal diameter, film thickener of 0.25 µm; the oven temperature program adopted was 40°C (5 min) with an increase of 5°C/min until 310°C (1 min). Mass spectra were recorded at 70 eV, Manifold 40°C, Ion Source temperature 280°C, Transfer line 320°C, Acquisition: Full Scan 50-800 uma. Most of the compounds were identified. GC-MS retention indices (authentic chemicals), and mass spectra (authentic chemicals and NIST and/or the WILEY spectral library collection) (Apostolides *et al*., 2013).

Statistical analysis

Statistical analysis was performed using the ANOVA (Tukey-Kramer). The difference between the average was considered statistically significant at the 5% level ($P < 0.05$).

Result

Secondary metabolites identified in the trunk bark of *D. oliveri*

The trunk bark of *D. oliveri* contains (table1) saponins, mucilages, catechol tannins, polyphenols, flavonoids, alkaloids, leuco anthocyanins, reducing compounds, sterols and terpenes.

Table1.Metabolitesidentified in trunkbark of *D. oliveri*

Secondary metabolites	
Phenols	+
Flavonoids	+
Alcaloids	+
Anthocyanins	-
Tannins	+
Coumarins	-
Saponosids	+
Sterols and terpenes	+
Reducing compound	+
Anthraquinones	-
Leuco-anthocyanins	+
Quinones	-
mucilage	+
volatile compounds	-

+: Presence; -: Absence

Extraction Yield

The ethanol extract (table 2) of the trunk bark of *D. oliveri* showed the lowest extraction yield (22.35 ± 4.03%) while that of the hydro hydroethanolic presented the most high efficiency (32.90 ± 8.34%). The yield for the aqueous extract was (23.10 ± 2.97) %. Statistically, this variation efficiency is not significant (P <5%).

Table2. Extraction yields

Extracts	yields (%)
Ethanolic	17.40±1.98[a]
Hydroethanolic	32.90±8.34[a]
Aqueous	23.10±2.97[a]

Values are mean ±SD (n=3). Means with the same letters in the same column are not significantly different (p<5%).

Phenolic compounds content of the crude extracts and fractions

The phenolic compounds contents of the extracts of the trunk bark of *D. oliveri*are shown in Table 3.
The total phenols content of the ethanol extract was (3.605±0.057) mg EAG/gDM while those of hydroethanolic and aqueous extract were respectively (3.986±0.242) mg EAG/gDM and (5.335±0.758) mg GAE/g D M.
Regarding condensed tannins content, ethanol extract has the highest value (290.233 ± 33.152 mg EC / GMS), followed by those of hydroethanolic (217.302±1.579 mg EC/g MS) and aqueous extracts (150.326 ± 0.789 mgCE /gMS).

Table 3. Phenolics compounds contents of *D. oliveri* crude extracts and fractions

Crudeextracts	TP (mg GAE/gDM)	TF (Mg CE/gDM)	CT(mg CE /gDM)
Ethanolic	3.61±0.06[a]	82.07±0.55[a]	290.23±33.15[a]
Hydroéthanolic	3.99± 0.24[a]	(79.81±1.28)[a]	217.30±1.58[a]
Aqueous	5.34±0.76[a]	84.09±0.34[a]	150.33±0.79[a]

TP: Total phenols; TF: Total flavonoids; CT: condensed tannins

Values are mean±SD (n=3). Means with the same letters in the same column are not significantly different (p<5%).

Radical scavengingactivity of extracts and fractions of the trunk bark of D. *oliveri*

Figures 2 and 3 show the scavenging percentage of DPPH radical based on concentrations of crude extracts and fractions of D. oliveri trunk bark. There is an increase of the scavenging percentage which becomes constant at concentrations that vary according to the nature of the extract. There is also a sudden increase in scavenging percentage with increasing concentrations of the extracts before become almost constant. These curves allowed the determination of the concentration of the extracts and fractions for scavenging 50% (IC_{50}) of free radicals (Table 4). The IC_{50} of ethanol, aqueous and hydroethanolic extracts of the trunk bark of *D. oliveri* were respectively 2.5mg / mL, 6.5µg / mL and 6.5µg / mL. They were 0.25mg / mL for the ethyl ether fraction, 0.06g / mL and 3.5 mcg / mL for those of ethyl acetate and butanol fractions.

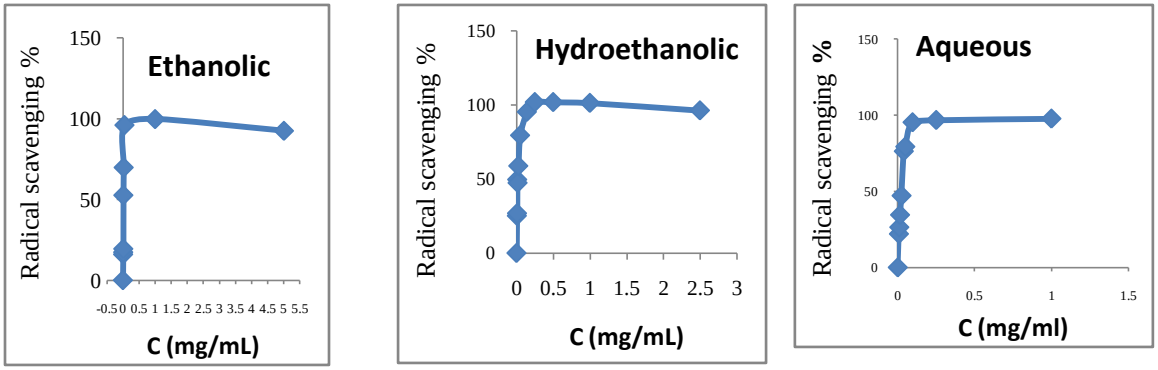
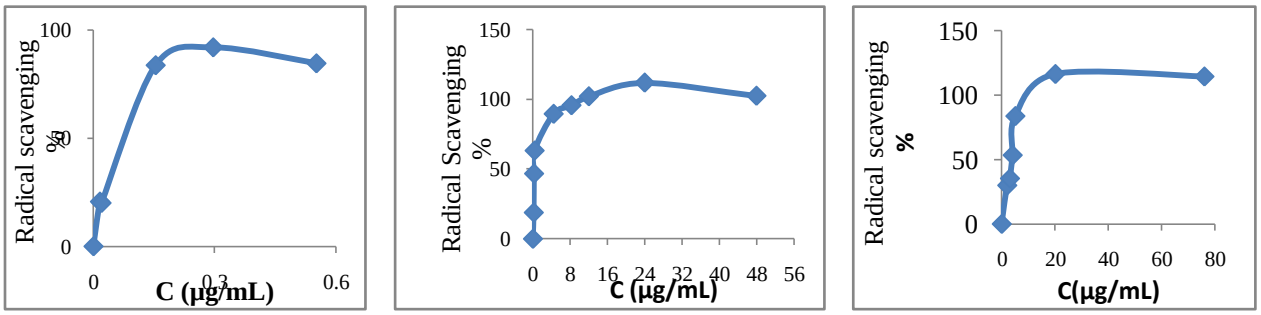


Figure 2. Antiradical activity of the crude extracts of *D. oliveri*



Ether ethylic Acetate Butanolic

Figure 3. Antiradical activity of the fractions of *D. oliveri* extract

Table4. IC_{50} values of crud extracts and fractions of the trunk bark of *D. oliveri*

Crude extracts	IC_{50} (µg/ml)	Fractions	IC_{50} (µg/ml)	BHA	Q	AG
Ethanolic	2.5	Ethyl ether	0.06	4.8	3	0.9
Hydroethanolic	6.5	Ethyl acetate	0.25			
Aqueous	6.5	Butanolic	3.5			

Antibacterial activity of crude extracts and fractions of *D. oliveri*

Analysis of Table 5 showed that crude extracts of *D. olivery* plant have bactericidal activity against *E. coli.* On *K. pneumoniae,* only ethanol and hydroethanolic extracts was bactericide. The aqueous extract inhibited only this strain at a concentration of 50mg / mL.Ethyque ether and butanol fractions have antibacterial activity against *E. coli* proved then only the ethyl ether fraction was bactericidal against *K. pneumoniae* strain.

Table5. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentration of crudeextracts and fractions

Bacteria	crudeextracts	Concentrations (mg/ml)		Fractions	Concentrations (mg/ml)	
		MIC	MBC		MIC	MBC
E. coli	Ethanolic	0.39	0.78	Ethylether	50.00	100.00
	Hydroethanolic	3.13	6.25	Ethylacetate	12.50	>100,00
	Aqueous	0.39	1.56	Butanolic	12.50	100.00
K. pneumoniae	Ethanolic	50.00	100.00	Ethylether	12.50	100.00
	Hydroethanolic	50.00	100.00	Ethylacetate	12.50	>100.00
	Aqueous	50.00	>100.00	Butanolic	50.00	>100.00

Chemical composition of the hydroethanolicextract of trunkbark of *D. oliveri*

The analysis of the chromatogram profile of the ethanolic extract of trunk bark of *D. oliveri* showed twenty two (22) compounds including glycerol ethanoate, pyrocatechol and Octadeca-9-enamide as major components (Table 6).

Table 6. Chemical composition of the hydroethanolic extract of D. Olivery trunk bark

RT (min)	Molecules
16.730	Glycerol ethanoate
19.756	Pyrocatechol
44.952	Octadeca-9-enamide

4.815	Methyl ethanoate
10.367	Oxime benzyloxymethyl
17.156	Valproic acid
22.454	Pyrocatechol 3-Méthyl
23.895	Syringole
25.283	Orthovanilline
30.629	Phloroglucinol
31.264	4-ethoxymethyl-2-methoxyphenol
31.746	α- methyl mannofuranoside
34.767	2-hydroxy-5-methylisophthalaldehyde
37.838	3-deoxyestradiol
41.867	Hexadecanamide
44.844	9Z-12-hydroxyoctadecenamide
51.847	Cholesta-2.4-diene
52.245	Cholestane
54.588	Ethylisoallocholate
55.477	Hydrocortisone acetate
57.747	carotenoid
58.330	ursodeoxycholic acid

Discussion

Comparaison of secondary metabolites identified in the trunk bark of D. *oliveri* with previous work showed divergences. For example, Kabore *et al*. (2010) reported the absence of alkaloids in the trunk bark of *D. oliveri* harvested in Burkina Faso. Mucilage and buffy anthocyanins identified in the trunkbark of *D. oliveri* harvested in Benin have been substituted by anthocyanins in Nigeria'ssample (Jegede et al., 2006). Tittikpina *et al*. (2013) identified anthraquinone in the sample of *D. oliveri* harvested in Togo that are absent in the sample of Benin. Our results were similar to those obtained by Adama (2009) in Burkina Faso. Kabore *et al*. (2010) reported the absence of alkaloids in the trunk bark of *D. oliveri* collected in the same country. This variability may be due to the period of harvest, the soil or climate factors (Daddona *et al*., 1976; Manolaraki, 2011). The diversity of secondary metabolites (mainly tannins and flavonoids) in *D. oliveri* could justify its use in veterinary medicine for the treatment of gastro intestinal diseases (Dicarlo *et al*., 1999).

Muanda (2010) obtained a total phenol content of 76 mg GAE/g DM in Mali. This value is very high compared to that of our results. As regards to the content of total flavonoids, the ethanolicextract showed a value of (82, 068 ± 0.548) mg CE/gDM, while those of the hydroethanolic and aqueous extract were (79.806±1.279) mg CE / g DM and (84.087±0.343) mg CE/gDM respectively. The total flavonoid content of the trunk bark of *D. oliveri* is insensitive to increase of the extraction solvent polarity. A total flavonoid content of 120mg EC/gDM in the samplefrom the trunkbark of this plant collected in Mali wasobtained by Muanda (2010). This content is higher than that noted in the Benin sample. This significant difference could be explained by the type of soil, the state of maturity and harvest time (Fallehe*t al.*, 2008).

About condensed tannins content, increasing of the polarity of the extraction solvent seems to have no effect on the content of total phenols and total flavonoids, but it influences the content of condensed tannins in the 5% level. The variability of levels of plant secondary metabolites according to region may depend to several factors (climate and soil conditions temperature, sun exposure, drought, salinity, storage conditions and the state of maturity of the plant) (Podsedek, 2007; Falleh*et al.*, 2008).

On the other hand, values fromantiradical potential of *D. oliveri* showed that the ethyl ether fraction was the most active, followed by ethyl acetate. The antiradical activities of these fractions were more pronounced than those of butylhydroxyanisole, quercetin and gallic acid, which are synthetic compounds used as reference in this study. In addition, *D. oliveri* extracts had bactericidal activities on tested microorganisms. These results are similar to those of Muanda (2010) who showed that *E. coli* has a pronounced sensitivity to the extracts of the trunk bark of *D. oliveri* from Mali. These results were also in accord with those of Ahmadu *et al*. (2003), Iwueke *et al*. (2008) and Yahaya *et al*. (2011) who showed in their study the bactericidal activity of the extracts of the trunk bark of *D. oliveri* against strains of *E. coli* and *K. pneumoniae*.

The hydroethanolic extract of the trunk bark of *D. oliveri* was richer in compounds which could justify the biological properties observed. Brand-Willams *et al*. (1995) have shown that phenols have anti-radical activity more effective than monophenols, for example, gallic acid, triphenolis more active than the protocatechic acid. These researchers have also shown that the compounds having the hydroxyl group in the ortho and para positions have a very strong anti-radical activity in comparison with those having a hydroxyl group in the meta position. Almela *et al*. (2006) showed that the effective anti-radical activitydepends on several factors such as the concentration of phenolic compounds, isometricforms and the synergistic interaction withother components. Referring to thisearlierwork, we could deductthat the difference of anti-radical activity noticed between the extracts and fractions of *D. oliveri*couldbe due to the presence of those compounds. The presence of phenolic compounds in extractscouldalsoexplain the bactericidalactivity of D. olivery (Boissier*et al*., 1993).

Conclusion

This study revealed that *Daniellia oliveri*, plant used in Benin for treatment of gastrointestinal diseases of livestock, contained secondary metabolites such as saponins, mucilage, catechin tannins, polyphenols, flavonoids, alkaloids, leuco-anthocyanin, reducing compounds, sterols and terpenes. Glycerol ethanoate, pyrocatechol and octadeca-9-enamide were the major compounds identified in the stem bark of this plant by Gas Chromatography. This plant showed an antiradical activity more interesting than those of butylhydroxyanisole, quercetin and gallic acid, and was bactericidal against *E. coli* and *K. pneumoniae*. These biological properties could be justified by the chemicals compounds identified and underpin the use of this plant in veterinary pharmacopoeia. It is therefore appropriate to orient future studies to evaluate the toxicity and make a bio-guided fractionation of extracts of this plant.

Acknowledgement

The authors of thisworkthank the rectoral team of University of Abomey-Calavi for hisfinancial support through the project “PHARMAVET". The authors of this publication thank Mr Faustin Aïssi, Emeritus Professor Holder of the University of Lille 1 (France), which facilitated collaboration between our laboratory and "l’Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV) de la Maison de Recherche sur l’Environnement Industriel (MREI) de l’Université du Littoral Côte d'Opale". This active collaboration has facilitatedchromatographicanalyzes.

References

1. Faye B, Le rôle de l’élevage dans la lutte contre la pauvreté. Revue d’élevage et de médecine vétérinaire des pays tropicaux, 54, 231-238, 2001.
2. [Tamboura, H., Kabore, H., and Yameogo, S. M, Ethnomédecine et pharmacopée vétérinaire traditionnelle dans le plateau central du Burkina Faso: cas de la province du Passoré. Biotechnol. Agron. Soc. Environ. 2(3):181-191, 1998
3. Anderson M, Marshall R, Dickson J, Prediction of bacterial penetration into carcass tissue during washing. In: Proc. 37th ICMST, september 1-6, Kulmback, Germany, Federal Center for Meat Research, Kulmback, Germany. 2, 1991;
4. FAO, Ticks and tick-borne disease control. A practicalfieldmanual. (Contrôle des tiques et des maladies qu'ils véhiculent. Un guide pratique sur le terrain). Rome, FAO, 1984, pp. 621.

5. Uilenberg G, A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. (Un guide de terrain pour le diagnostique, le traitement et la prévention de la trypanosomiase animale africaine). Rome, FAO, 1998, pp. 158;

6. Arnaud S, Phytothérapie en médecine vétérinaire des animaux de compagnie. La phytothérapie européenne, (53), 12-14, 2009;

7. Baerts, M., Lehmann J, Baerts (Martine) & Lehmann (Jean), Plantes médicinales vétérinaires de la région des crêtes Zaïre-Nil au Burundi. Tervuren, Musée Royal de l'Afrique Centrale (MRAC), 1991, 133 p;

8. BÂ A.S, L'art vétérinaire et la pharmacopée traditionnelle en Afrique sahélienne Rev.sci. tech. Off. int. Epiz, 13(2):373-396, 1994;

9. Anon. Committee to assess agro residues availability.INPAPER International 1(1):13, 1996.

10. Byavu, N., Henrard, C., Dubois, M., &Malaisse, F, Phytothérapie traditionnelle des bovins dans les élevages de la plaine de la Ruzizi.Biotechnologie, Agronomie, Société et Environnement, 4(3), 135-156, 2000;

11. Dassou H.G, Ogni, C.A, Yedomonhan, H., Adomou A.C., Tossou, M., Dougnon, J.T et Akoegninou A, Diversité, usages vétérinaires et vulnérabilité des plantes médicinales au Nord-Bénin. Int. J. Biol. Chem. Sci. 8(1): 189-210, 2014;

12. Ogni, C.A., Kpodekon, M. T., Dassou, H.G., Boko, C.K., Koutinhouin, B. G., Dougnon, J.T., Youssao, A.K.I, Yedomonhan, H. et Akoegninou A, Inventaire ethno-pharmacologique des plantes utilisées dans le traitement des pathologies parasitaires dans les élevages extensifs et semi-intensifs du Bénin. Int. J. Biol. Chem. Sci. 8(3): 1089-1102, 2014.

13. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Technique & Documentation. Paris. 1999;

14. Soro, T.Y., Traore, F., Datte, J.Y. and Nene-Bi, A.S, Antipyretic activity of aqueous extract of Ximenia americana, Phytoterapie, 7(6):297-303, 2009.

15. Dohou, N., Tahrouch, K.Y, Idrissi S.,,Hassani, L.M, Bodoc A., Gmira, N, Screening phytochimiqued'uneendémieIbéro-marocaine, Thymelaealytroides. Bull. Soc. Pharm. Bordeaux, 142: 61-78; 2003.

16. Békro, Y. A., Békro J. A. M., Boua, B. B., Tra, B.F.H, Ehilé, E.E, Etude ethnobotanique et screening phytochimique de Caesalpiniabenthamiana (Baill.) Herend et Zarucchi (Caesalpiniaceae). Re. Sci. Nat, 4(2): 217-225, 2007;

17. Boissier Z.R, Asselimean J, Zalta J.P. Les antibiotiques, structures exemples de mode d'action, Herman, Paris, 1993.

18. Ziegler J. L'empire de la honte (p. p173). Paris: Fayard, 2005