

RESEARCH

Open Access



In vitro antibacterial, non-cytotoxic and antioxidant activities of *Boscia Senegalensis* and *Tapinanthus dodoneifolius*, plants used by pastoralists in Cameroon

Ronald Romuald Bebey Vougat Ngom^{1*} and Harquin Simplicie Foyet²

Abstract

In the Far North Region of Cameroon, pastoralists use the leaves of *Boscia senegalensis* and the stem of *Tapinanthus dodoneifolius* to treat common animal diseases. This study aimed to evaluate the in vitro antibacterial, non-cytotoxic and antioxidant potentials of these plants. To achieve this, four extracts (water, methanol, chloroform and hexane) of both plants obtained by successive fractionation were used. Antibacterial activities of the different extracts were evaluated against three bacterial reference strains including Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) using agar disc diffusion and broth dilution methods. Human colon cancer cells were used to screen their toxicity. 2,2-Ddiphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferrous ion chelating assays have been used to investigate the antioxidant activities of the best extract of each plant after antibacterial assay. A sensitive inhibitory effect was observed against *S. aureus* with hexane extract of *B. senegalensis* and methanolic extract of *T. dodoneifolius*. In addition, the results showed that both plant extracts are not toxic. The hexane and methanolic extracts of *B. senegalensis* and *T. dodoneifolius*, respectively, showed higher antioxidant activities, but the hexane extract demonstrate a strong hydrogen donating ability or the electron transfer reaction in comparison with vitamin C used as standard. This finding may support the traditional use of both plants for managing animal diseases in the Far North of Cameroon.

Keywords: Antibacterial, Cytotoxic, Antioxidant, *Boscia senegalensis*, *Tapinanthus dodoneifolius*, Extract, Cameroon

Introduction

In many African countries where the livestock sector plays a prominent role in the economy especially for the rural population, animal diseases constitute one of the main hindrances (Mthi et al. 2018; Bolajoko et al. 2020). Animal diseases limit livestock production and productivity with significant impacts on animal health, public health and economies (McElwain and Thumbi 2017; Chakale et al. 2021). Indeed, in these regions, livestock act as a “bank” for the provision of cash derived from

sales of their products or of the animals themselves in times of crisis, to raise funds needed to purchase food and meet other family needs.

In some areas of those countries (i.e. South Africa, Far North of Cameroon), because of the lack of access to veterinary drugs, and their mobility, many pastoralists use traditional medicines for preventing and managing animal diseases (Chitura et al. 2018). In fact, this traditional practice is continuing because pastoralists believe that medicinal plants are efficient, available and easily accessible and animal health is a prerequisite for higher productivity (FAO (Food and Agriculture Organization of the United Nations), 2013; Vougat et al. 2015). Researchers are working on this subject matter to actually

* Correspondence: romsonbey@yahoo.fr

¹Department of Animal Production, School of Veterinary Medicine and Science, University of Ngaoundere, Ngaoundere, Cameroon
Full list of author information is available at the end of the article

confirm the potential of the plants used by livestock farmers (Mostafa et al. 2018; Nordin et al. 2019). The increasing focus on natural products might be due to their reduced or non-cytotoxicity or low side effects compared with chemically synthesized drugs (Hyun et al. 2013). In addition, plant extract usage represents an important alternative for the reduction of the use of drugs like antibiotics, known to be the main trigger of the development and spread of antimicrobial resistance, a major global threat to human health (Dadgostar, 2019; Varona et al. 2020). That is especially important for developing countries like Cameroon where stringent policies on antibiotic use are not fully in place (Mouiche et al. 2018).

Unfortunately, in Cameroon where livestock contributes significantly to the gross national product (MINEPIA 2009), no sufficient attention is focused on ethnoveterinary medicine. It is common knowledge that in the Far North region of Cameroon, the region that has the second highest cattle population in the country, many cattle farmers and specially pastoralists treat their animals with plant extracts (Vougat et al. 2015; MINEPIA, 2019 unpublished). Due to the increasing importance of such medicine to cattle farmers, our research team seeks to analyse the plants used to control and treat animal diseases. Our previous study in the Far North region of Cameroon consisted of investigating the plants used against one of the most common, frequent and recognizable infectious diseases (Foot and Mouth Disease or FMD) and evaluating the phytochemical composition and antioxidant potentials of the two mostly used (*Boscia senegalensis* and *Tapinanthus dodoneifolius*) (Vougat et al. 2015). Thus, this study aims to deepen the scope of analysis on these two plants which have a good phytochemical profile (Vougat et al. 2015; Idu et al. 2016). Both plants are also used in many countries to treat animal and human diseases (FMD, respiratory tract infections, stomach ache, diarrhoea, dysentery, diabetes, epilepsy, hepatitis in humans) (Vougat et al. 2015; Mamman et al. 2019; Mohamed et al. 2020a).

Therefore, with due knowledge of their profile and immense importance, this study was designed to evaluate the antibacterial, non-cytotoxic and antioxidant potentials of *B. senegalensis* and *T. dodoneifolius*. Antimicrobial activity is first screened because antibiotic drugs are the main veterinary medicine used by pastoralists in the study area (Vougat Ngom et al. 2017). The cytotoxic activity was evaluated to verify the safety of these plants.

Study area

The plants analyzed in this study were collected in the Far North region of Cameroon as previously described (Vougat et al. 2015). In Cameroon, pastoralists are mainly located in three regions (North West, Adamawa

and Far North regions) (Kelly et al. 2016; Motta et al. 2018) where more than 80% of the national cattle, sheep and goat population are found (MINEPIA, 2009 Not published). However, due to its climate conditions, the Far North region has the majority of pastoralists of the country. This region is characterized by a Sudano-Saharan climate. The average annual rainfall in this region is about 700 mm. The temperatures are generally low in the rainy season and the nights in the dry season between December and January (Dassou et al. 2015).

Methods

Preparation of plant extracts

T. dodoneifolius was collected from the host plant *Acacia albida* as a study has shown that the properties of *T. dodoneifolius* depended on its host plant (Idu et al. 2016). These plants were then identified and authenticated in the National Herbarium in Yaoundé (Cameroon) where the voucher specimens already existed under the reference numbers 23137 SRF/Cam and 50271 HNC respectively for *B. senegalensis* and *T. dodoneifolius*. The extraction of both plant samples was done according to the scheme below (Fig. 1). Each partition was obtained after washing three times with the solvent. Each dried fraction was kept in a freezer before biological tests.

Antibacterial assay

The antibacterial activity of both plants was evaluated by using both qualitative and quantitative methods.

Selected test bacteria

Three bacterial strains were used for the study: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) and an in house fully characterized *Salmonella typhimurium*. These bacteria are among the most common foodborne bacteria in animal source food that cause a variety of diseases in humans and animals (Rortana et al. 2021). Bacterial strains were grown and maintained on Trypticase Soy Agar with 5% Sheep Blood plates (BD BBM™ Becton, Dickinson and Company, Sparks, USA). Bacterial suspensions of 0.5 McFarland

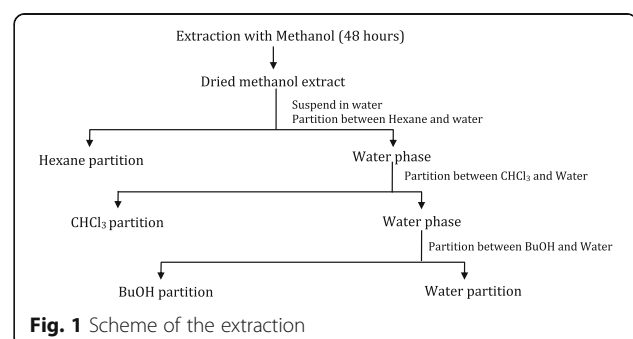


Fig. 1 Scheme of the extraction

were prepared with sterile saline solution to inoculate plates during the antibacterial assay.

Extracts re-suspension and paper disc preparation

Sterile saline solution was used to dissolve extracts of *B. senegalensis* obtained with methanol and water. For the other extracts (hexane and chloroform extracts of *B. senegalensis* and all the *T. dodoneifolius* extracts), 15% dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific Inc., Pittsburgh, USA) was used (Kawo et al. 2011; Kpadonou-Kpoviessi et al. 2013). Each extract was prepared to yield a stock concentration of 100 mg/ml. After dilution of the stock solution, seven final concentrations (0.001, 0.01, 0.1, 1, 5, 10 and 20 mg/ml) of each sample were used to be tested against a bacterial suspension. Sterile filter paper discs of 6 mm in diameter (BD BBM™ Becton, Dickinson and Company, Sparks, USA) were impregnated with 50 µl of each concentration to give a respective final amount of 5×10^{-5} , 5×10^{-4} , 5×10^{-3} , 5×10^{-2} , 0.25, 0.5 and 1 mg/disc. A sterile paper disc prepared in the same condition with 50 µl of only solvent used for the corresponding extract was used as a negative control. Discs were allowed to dry at 37 °C for 24 h (so as to remove residual solvent, which might interfere with the determination).

Agar disk diffusion assay

Antimicrobial activities of the different extracts were screened for their inhibitory zone by the agar disc diffusion method as described elsewhere (Gautam et al. 2013). Briefly, Mueller Hinton Agar medium plates (BD Difco™ Becton, Dickinson and Company, Sparks, USA) were inoculated with a bacterial suspension at 0.5 McFarland, and discs with extract were placed on the surface of the agar. Plates were incubated at 37 °C for 24 h. The results were recorded by measuring the zone of growth inhibition (mm) surrounding the discs. Each concentration was tested in duplicate. The results of the diameters of the zones of inhibitions of extracts were interpreted as sensitive (> 18 mm), intermediate (14–17 mm), and resistant (< 14 mm) (Mohamed et al. 2020b).

Broth dilution method

The minimum inhibitory concentration (MIC), which is considered as the lowest concentration of the sample which inhibits the visible growth of a microbe, was determined by the broth dilution method. A dilution method (Elof 2004) was followed for the determination of MIC values with few modifications. Briefly, for each extract, different volumes of the stock solution were added to tubes containing different volumes of Trypticase Soy Broth (BD BBM™ Becton, Dickinson and Company, Sparks, USA) to obtain the concentrations of 0.001, 0.01, 0.1, 1, 5, 10 and 20 mg/ml. Thereafter, 20 µl

inoculum (for bacteria 1×10^5 CFU/ml) was added to each tube. A tube containing 300 µl of TSB, 200 µl of solvent used for extract dilution and 20 µl inoculum was used as a negative control. Each concentration was assayed in duplicate. All the tubes were then incubated at 37 °C for 24 h. After incubation, the tubes were then examined for microbial growth by observing for turbidity. The MIC values were taken as the lowest extract concentration that showed no turbidity after incubation.

Cytotoxicity activity

Human colon cancer cells (HT-29) were obtained from the American Type Culture Collection (ATCC catalogue no. HTB-38). Colon cancer cells were cultured in RPMI 1640 medium (Hyclone) containing 100 µg/ml streptomycin, 100 units/ml penicillin, 0.25 µg/ml amphotericin B (Fungizone) and 10% fetal bovine serum (FBS). The cell line was cultured at 37 °C in a humidified incubator of an atmosphere of 95% air and 5% CO₂. Cells were trypsinized and split for subculture when they reached near-confluent state (5 days). Cells are typically grown to 60–70% confluence, the medium was then changed and the cells were used for test procedure 1 day later according to the method described by Skehan et al. (1990) and Pan et al. (2010).

Antioxidative assay

The antioxidant assay was evaluated for the extracts that showed good results during antimicrobial and cytotoxicity analyses. The experiments were conducted in triplicate.

DPPH radical scavenging activity

The antioxidant activities of the samples were measured by determining the radical scavenging ability using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as described by Tepe et al. (2005). The activity of each extract was represented by the IC₅₀ parameter (substrate concentration required to cause the loss of 50% of the initial concentration of DPPH), and vitamin C was used as standard.

Ferrous ion chelating capacity

The chelation of iron ions (II) was studied as described by Suter and Richter (2000) with minor modifications. The reaction solution containing 100 µl (2 mM) ferrous chloride and 400 µl (5 mM) potassium ferricyanide as reagent was prepared. A 200-µL test sample of the extract at various concentrations ranging from 2 to 10 mg/mL was prepared in different test tubes. Double distilled water was added to each test tube to a 1-ml level and mixed. The above reagent was then added, and the reaction mixture was incubated at 20 °C for 10 min. The formation of the potassium hexacyanoferrate complex was

measured at 700 nm using a spectrophotometer. The assay was carried out at 20 °C to prevent Fe²⁺ oxidation. Lower absorbance indicated a higher iron chelating capacity. The negative control was without any chelating compound or test sample of extract. Ethylenediaminetetraacetic acid (EDTA) used as a control was prepared in the same way as the test samples and treated with the same reagent. The per cent of ferrous ion chelating capacity was calculated accordingly by comparing the absorbance of the test samples with that of the negative control.

$$\text{Ferrous ion chelating capacity} = [(A_{\text{control}} - A_{\text{extract}})/A_{\text{control}}] \times 100$$

A_{control} is the absorbance of the negative control, and A_{extract} is the absorbance of the extract solution.

Statistical analysis

For the antioxidant activity of the plant extracts, data were presented as mean \pm standard deviation. One-way analysis of variance followed by Duncan's multiple range test was performed using Statgraphics 5.0. (Windows, www.statgraphics.com) to compare these data. But for the antimicrobial assay where a comparison was not needed, MIC are presented as mean. The IC₅₀ (median growth inhibitory concentration) values of test samples for the cytotoxicity assay in serial dilutions were calculated using non-linear regression analysis (Table curve2Dv4; AISN Software, Inc., Mapleton, OR). The significance level was fixed at 0.05 for all the statistical analyses.

Results

Antibacterial activity of plant extracts

Tables 1 and 2 show the results of MIC determination of the test bacteria. At all the concentrations, chloroform, methanol and water extracts of *B. senegalensis* did not inhibit the growth of the bacteria studied. The same result was observed for all the extracts of *T. dodoneifolius* at 1, 0.1, 0.01 and 0.001 mg/ml. A conclusion of the bacterial growth in the tubes containing the hexane extract of *B. senegalensis* at 20, 10 and 5 mg/ml was not clear because before adding inoculum, the tubes were

turbid. Therefore, this was not easy to know if they were the growth of bacteria or not. This was also observed in the tubes containing *S. aureus* and water or methanol extracts of *T. dodoneifolius* at 20, 10 and 5 mg/ml. The same results were found in the tubes containing *S. aureus* and hexane or chloroform extract of the same plant at 20 and 10 mg/ml.

The summarized results of the antibacterial susceptibility test of *B. senegalensis* and *T. dodoneifolius* against the test bacteria are presented in Tables 3 and 4, respectively. Hexane extract of *B. senegalensis* was the most effective, and the highest activity was demonstrated at 20 mg/ml (16 mm zone of inhibition), followed by 10 mg/ml (14.75 mm) and 5 mg/ml (13.25 mm) against Gram-positive bacteria. The methanol extract of *T. dodoneifolius* shows the highest activity against the same microorganism at 20 mg/ml (10 mm).

Cytotoxicity assay of the plant extracts

Four extracts of each plant sample were evaluated for their cytotoxicity against human colon cancer cells (HT-29) in vitro, and the results are presented in Table 5. All the extracts showed an IC₅₀ significantly higher than 40 μ g/ml.

Antioxidant analysis

The antioxidant capacity of the extracts studied is shown in Table 6. From this table, it appears that the scavenging activity extract of *B. senegalensis* (28.80 \pm 1.18 μ g/ml) is significantly higher than that of vitamin C (107.65 \pm 3.72 μ g/ml) and *T. dodoneifolius* (767.28 \pm 2.78 μ g/ml).

The chelating of Fe²⁺ by extracts was estimated, and the extent to which an extract can form complexes with the ferrous ion reflects its antioxidant activity. The IC₅₀ of EDTA (0.50 \pm 0.12 μ g/ml) was significantly lower than that of the hexane extract of *B. senegalensis* (3.36 \pm 0.10 μ g/ml).

Discussion

The determination of the MIC of *B. senegalensis* showed that the chloroform, methanol and water extracts at all concentrations have no activity against the bacteria studied. The same result was found by Aliyu et al. (2008).

Table 1 Minimum inhibitory concentration (MIC) of *B. senegalensis* on the test bacteria

Microorganism	Concentrations of the extract (mg/ml)/MIC (mm)															
	20				10				5				≤ 1			
	M	H	C	W	M	H	C	W	M	H	C	W	M	H	C	W
<i>S. aureus</i> (ATCC 29213)	+	/	+	+	+	/	+	+	+	/	+	+	+	+	+	+
<i>E. coli</i> (ATCC 25922)	+	/	+	+	+	/	+	+	+	/	+	+	+	+	+	+
<i>S. typhi</i> (14028)	+	/	+	+	+	/	+	+	+	/	+	+	+	+	+	+

M methanol, H hexane, C chloroform, W water

"+" = growth of bacteria; "/" = turbidity of the tube was difficult to read because of the presence of two phases, the upper phase was very clear and the second dense as before the introduction of the inoculum

Table 2 Minimum inhibitory concentration (MIC) of *T. dodoneifolius* on the test bacteria

Microorganism	Concentrations of the extract (mg/ml)/MIC (mm)															
	20				10				5				≤ 1			
	M	H	C	W	M	H	C	W	M	H	C	W	M	H	C	W
<i>S. aureus</i> (ATCC 29213)	/	/	/	/	/	/	/	/	/	+	+	/	+	+	+	+
<i>E. coli</i> (ATCC 25922)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. typhi</i> (14028)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

M methanol, H hexane, C chloroform, W water

"+" = growth of bacteria; "/" = turbidity of the tube was difficult to read because of the presence of two phases, the upper phase was very clear and the second dense as before the introduction of the inoculum

However, this is in contradiction with Prashant et al.'s (2011) review. Indeed, according to them, these tree solvents are used for the extraction of antibacterial compounds in plant materials. However, the results of the hexane extract at 20, 10 and 5 mg/ml were difficult to be appreciated.

The results of the antibacterial activity of both plants showed that only one extract of each plant (hexane extract of *B. senegalensis* and methanol extract of *T. dodoneifolius*) has a significant activity against the strain of Gram-positive bacteria *S. aureus*. As this plant is commonly used against FMD in Far North of Cameroon, this result could suggest that the opportunistic bacterial diseases that affect animals infected by FMD could be due to Gram-positive bacteria. This is in correlation with the fact that the main veterinary drugs used by the pastoralists in this study area to manage cattle infected by the said disease consist of penicillin G (Vougat Ngom et al. 2017), an antibiotic belonging to the class with the action spectrum encompasses mainly Gram-positive bacteria (Lobanovska and Pilla 2017).

B. senegalensis activity against *S. aureus* demonstrated in this work is similar with the results found by Aliyu et al. (2008) who showed its activity against *S. aureus* resistant to methicillin, an antibiotic belonging to the class of penicillins M. This coherence of results could support the hypothesis that the hexane extract of *B. senegalensis* contain molecules which have the same mechanism of action like penicillins. This argument could be supported by the proximity between the diameter of the inhibition zone (16 mm) of *B. senegalensis* found in this work and those obtained by the authors cited above (19 mm). The similarity in the phytochemical composition of the

extracts used during the study of Aliyu et al. (2008) is their compositions in tannins, saponins and alkaloids, compounds known to have antibacterial properties (Prashant et al. 2011). Taking into consideration the antioxidant properties found and the properties of the organic solvent used for the extraction, the antibacterial activity observed can be the result of those compounds.

The antibacterial activity of the methanol extract of *T. dodoneifolius* against *S. aureus* is similar with the finding of Deeni and Sadiq during the study they conducted in Northern Nigeria (Deeni and Sadiq 2002). This similarity could be justified by its high content of phenolic compounds and other compounds known for their antibacterial activities (Tavassoli and Djomeh 2011). Contrary to our results, the work of Ndamitso et al. (2013) showed that this methanol extract has a low activity. This difference could be referred to the different parts of the plants studied and their host plants. Indeed it has already been shown that the properties of this hemiparasitic plant depend on its host (Deeni and Sadiq 2002).

The non-cytotoxic effect of the methanol, hexane, water and chloroform extract of *B. senegalensis* and *T. dodoneifolius* was evaluated in vitro to assure their safety. This potential has been demonstrated using the colon cancer cells (HT-29) and four concentrations of each extract. The results clearly showed that all the extracts have a very significantly higher IC₅₀ in comparison with 40 µg/ml. Taking into consideration the classification criteria of the extracts according to their cytotoxic potential as defined by the National Cancer Institute, we can say that apart from the hexane extracts of both plants and the aqueous extract of *T. dodoneifolius* considered moderately toxic, all the other extracts are not

Table 3 Antibacterial activity of *T. dodoneifolius* on the test bacteria

Microorganism	Concentrations of the extract (mg/ml)/zone of inhibition (mm)															
	20				10				5				≤ 1			
	M	H	C	W	M	H	C	W	M	H	C	W	M	H	C	W
<i>S. aureus</i> (ATCC 29213)	10	0	7	7.25	8	0	0	0	7	0	0	0	0	0	0	0
<i>E. coli</i> (ATCC 25922)	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. typhi</i> (14028)	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0

M methanol, H hexane, C chloroform, W water

Table 4 Antibacterial activity of *B. senegalensis* on the test bacteria

Microorganism	Concentrations of the extract (mg/ml)/zone of inhibition (mm)															
	20				10				5				≤ 1			
	M	H	C	W	M	H	C	W	M	H	C	W	M	H	C	W
<i>S. aureus</i> (ATCC 29213)	0	16	0	0	0	14.75	0	0	0	13.25	0	0	0	0	0	0
<i>E. coli</i> (ATCC 25922)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. typhi</i> (14028)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Values are the means of two assays

M methanol, H hexane, C chloroform, W water

cytotoxic. Indeed, according to this institute, an extract that inhibits the proliferation of cells with an $IC_{50} \leq 20 \mu\text{g/ml}$ is considered as an active anticancer extract, and an extract with $20 \mu\text{g/ml} < IC_{50} < 100 \mu\text{g/ml}$ is indicated as moderately. The extracts with an $IC_{50} > 100 \mu\text{g/ml}$ are inactive compounds (Tanamatayarat et al. 2003; Mutee et al. 2012). The non-toxic effect of different extracts of the plants studied may be due to their antioxidant activity as already highlighted by Kilani et al. (2008). This can justify the usage of these plants by people of various countries in Africa (Inuwa et al. 2012; Ndamitso et al. 2013; Bekoe et al. 2020).

The stable DPPH radical is widely used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts (Talla et al. 2014). This scavenging activity was revealed to be higher for *B. senegalensis* than that of vitamin C and *T. dodoneifolius*. However, the activity of hexane extract of *B. senegalensis* found here is less than that of the methanolic and water extract of the same plant obtained in our previous study (Vougat et al. 2015). This difference can be explained by the polarity of the solvents used for the extraction (Ncube et al. 2008). Indeed, the non-polar solvent (hexane) used showed less activity than the polar protic solvent (water and methanol). Putting together this result and our previous finding (Vougat et al. 2015), it could be suggested that the antioxidant activity of *B. senegalensis* involved its hydrogen donating ability or the electron transfer reaction (Foti et al. 2004; Friaa et al. 2008). Indeed, DPPH is a stable free radical and

accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al. 1997).

One of the assays of antioxidative action is chelation of transition metal, thus preventing catalysis of hydroperoxide decomposition and fenotype reactions (Soares et al. 1997). The hexane extract of *B. senegalensis* showed a significantly high chelating effect than *T. dodoneifolius*. This finding is not due to the solvent used because generally extracts of solvents of low polarity show lower chelating capacity while those of higher polarity show higher chelating capacity (Talla et al. 2014). It is certainly the result of the phytochemical composition of both extracts as also observed by Ho et al. (2012). Indeed, it is well known that chelating capacity is attributed to flavonoids and phenolic compounds which use their redox properties to chelate transition metals (Gülçinllhami et al. 2004). However, the fact that the extract of *B. senegalensis* demonstrated a less chelating effect than EDTA suggest that this plant is a moderate metal chelating agent as compared to this standard.

Conclusions

In this study, several *in vitro* assays were applied to evaluate some properties of different extracts of *B. senegalensis* and of *T. dodoneifolius*, two plants most used by pastoralists in the Far North Region of Cameroon. The findings showed that the plant extracts are no toxic. Some bacteria tested were highly sensitive to these extracts. The observed bioactivities of *B. senegalensis* and *T. dodoneifolius* may support the traditional use of both plants for managing animal diseases in the Far North of

Table 5 IC_{50} of *T. dodoneifolius* and *B. senegalensis* on human colon cancer cells (HT-29)

Extract	Plant samples	
	<i>T. dodoneifolius</i>	<i>B. senegalensis</i>
Methanol	121.74	176,330.92
Hexane	66.13	44.37
Chloroform	713,879.28	66,565.78
Water	68.02	103.48

Each value represents the IC_{50} of the extract of the plant

Table 6 IC_{50} ($\mu\text{g/ml}$) value of different extracts

	Antiradical activity on DPPH ^a	Ferrous ions chelating capacity ^b
Standard	107.65 ± 3.72 ^b	0.50 ± 0.12 ^c
<i>B. senegalensis</i>	28.80 ± 1.18 ^c	3.36 ± 0.10 ^b
<i>T. dodoneifolius</i>	767.28 ± 2.78 ^a	431.48 ± 1.11 ^a

Values are the mean ± E.S.M; $n = 3$; values with different letters in the same column are significantly different ($p < 0.05$). α : standard used is vitamin C ($R^2 = 0.9629$); β : standard used is ethylenediamineacetic acid ($R^2 = 0.9007$)

Cameroon. The therapeutic properties of these plants could be due to their antioxidant and antibacterial effects of their secondary metabolites. However, further studies are necessary to examine the *in vivo* effect of those extracts and to isolate the active compounds responsible for these pharmacological activities.

Abbreviations

IC₅₀: Median growth inhibitory concentration; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; MIC: Minimum inhibitory concentration; EDTA: Ethylenediaminetetraacetic acid

Acknowledgements

Cytotoxicity activity of plants studied was performed at the Division of Medicinal Chemistry and Pharmacognosy of the College of Pharmacy, Ohio State University (OSU). The antimicrobial assay was conducted at the Department of Veterinary Preventive Medicine of the same university. But antioxidant activity was performed at the University of Ngaoundere. We acknowledge Dr. Rebecca Garabed from OSU and Dr. Ziebe from the University of Maroua for the advice and Pr. Douglas Kinghorn, Dr. Jie Li, and Dr. Hee-Byung Chai from the College of Pharmacy at OSU (OH, USA) for the assistance during extraction and non-cytotoxic assay. The authors thank Drs. Armando Hoet and Joany C. Van Valen from the College of Veterinary Medicine at OSU for giving us the opportunity to work with them.

Authors' contributions

VN and FH designed the study. VN collected the sample in the field under the supervision of FH. VN performed the laboratory work, analyzed and interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by the US National Science Foundation under Grant DEB-1015908 via a fellowship from the Disease Ecology and Computer Modeling Laboratory (DECML) of the Ohio State University (Columbus, OH, USA).

Availability of data and materials

The datasets used/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Animal Production, School of Veterinary Medicine and Science, University of Ngaoundere, Ngaoundere, Cameroon. ²Department of Biological Sciences, University of Maroua, Maroua, Cameroon.

Received: 24 November 2020 Accepted: 29 November 2021

Published online: 15 March 2022

References

- Aliyu, A.B., A.M. Musa, M.S. Abdullahi, and A.O. Oyewale. 2008. Phytochemical and antibacterial properties of *Ludwigiasuf fruticosa* (Willd.) Oliv. ex. O. Ktze (Onagraceae). *International Journal of Pure and Applied Sciences* 2 (4): 1–5.
- Bekoe, E.O., C. Agyare, Y.D. Boakyie, B.M. Baiden, A. Asase, J. Sarkodie, H. Nettey, F. Adu, P.B. Otu, B. Agyarkwa, P. Amoateng, I. Asiedu-Gyekye, and A. Nyarko. 2020. Ethnomedicinal survey and mutagenic studies of plants used in Accra metropolis, Ghana. *Journal of Ethnopharmacology* 248: 112309. <https://doi.org/10.1016/j.jep.2019.112309>.
- Bolajoko, M.B.F., A.R. Van Gool, J.S. Peters, C.J. Martinez, and V.B. Dungu. 2020. Field survey of major infectious and reproductive diseases responsible for mortality and productivity losses of ruminants amongst Nigerian Fulani pastoralists [version 1; peer review: awaiting peer review]. *Gates Open Research* 4: 162. <https://doi.org/10.12688/gatesopenres.13164.1>.
- Chakale, M.V., M. Mulunda, and O.A. Adeyemi. 2021. Ethnoveterinary knowledge and biological evaluation of plants used for mitigating cattle diseases: a critical insight into the trends and patterns in South Africa. *Frontiers in Veterinary Science* 8: 891. <https://doi.org/10.3389/fvets.2021.710884>.
- Chitura, T., P.T. Muvhali, K. Shai, B. Mushonga, and E. Kandiwa. 2018. Use of medicinal plants by livestock farmers in a local municipality in Vhembe District, South Africa. *Applied Ecology and Environmental Research* 16 (5): 6589–6605. https://doi.org/10.15666/aeer/1605_65896605.
- Dadgostar, P. 2019. Antimicrobial resistance: implications and costs. *Infection and drug resistance* 12: 3903–3910. <https://doi.org/10.2147/IDR.S234610>.
- Dassou, E.F., A. Ombolo, G.E. Mboudou, S. Chouto, E.B. Ambomo, and J.M.A. Essi. 2015. Caractérisation de la variabilité spatio-temporelle des précipitations dans la zone soudano-sahélienne du Cameroun au cours des cinq dernières décennies. *Afrique Science* 11 (4): 331–348.
- Deeni, Y.Y., and N.M. Sadiq. 2002. Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausa land, Northern Nigeria. *Journal of Ethnopharmacology* 83 (3): 235–240. [https://doi.org/10.1016/S0378-8741\(02\)00244-1](https://doi.org/10.1016/S0378-8741(02)00244-1).
- ElofEloff, J.N. 2004. Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. *Phytomedicine* 11 (4): 370–371. <https://doi.org/10.1078/0944711041495218>.
- FAO (Food and Agriculture Organization of the United Nations). 2013. *World livestock 2013: changing disease landscapes*, 130. Rome: FAO Available at: <https://www.fao.org/docrep/019/i3440e/i3440e.pdf>. Accessed 30 Sept 2014.
- Foti, M.C., C. Daquino, and C. Geraci. 2004. Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH radical in alcoholic solutions. *The Journal of Organic Chemistry* 69 (7): 2309–2314. <https://doi.org/10.1021/jo035758q>.
- Friaa, O., V. Chaleix, M. Lecouvey, and D. Brault. 2008. Reaction between the anesthetic agent propofol and the free radical DPPH in semiaqueous media: kinetics and characterization of the products. *Free Radical Biology and Medicine* 45 (7): 1011–1018. <https://doi.org/10.1016/j.freeradbiomed.2008.07.001>.
- Gautam, K., P. Kumar, and A. Jindal. 2013. Evaluation of antimicrobial efficacy of flavonoids and alkaloids of *Andrographis paniculata* nees. *International Journal of Green Pharmacy* 7 (1): 5757–5761. <https://doi.org/10.4103/0973-8258.111617>.
- GülçinIhami, Ö., K. Irfan, O. Münir, and E.B. Mehmet. 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urticadioica* L.). *Journal of Ethnopharmacology* 90 (2-3): 205–215. <https://doi.org/10.1016/j.jep.2003.09.028>.
- Ho, S., Y. Tung, Y. Chen, Y. Zhao, M. Chung, and J. Wu. 2012. Antioxidant activities and phytochemical study of leaf extracts from 18 indigenous tree species in Taiwan. *Evidence-Based Complementary and Alternative Medicine* 8: 1–8. <https://doi.org/10.1155/2012/215959>.
- Hyun, T.K., K. Myeong-ok, L. Hyunkyung, K. Younjoo, K. Ju-Sung, and K. Euikyung. 2013. Evaluation of anti-oxidant and anti-cancer properties of *Dendropanax moribifera* Léveillé. *Food Chemistry* 141 (3): 1947–1955. <https://doi.org/10.1016/j.foodchem.2013.05.021>.
- Idu, M.M., O. Ovuakporie-Uvo, and M.J. Nwaokolo. 2016. Phytochemistry and microscopy of *Tapinanthus dodoneifolius* (DC) (Danser) (Santalales: Loranthaceae) (African mistletoes) from guava, rubber and orange host trees. *Brazilian Journal of Biological Sciences* 3 (5): 27–35. <https://doi.org/10.21472/bjbs.030503>.
- Inuwa, H.M., V.O. Aina, S. Ibrahim, and D.A. Ameh. 2012. Phytochemical screening and antibacterial activity of *Globimetulla browni* extracts during dry season. *British Journal of Pharmacology and Toxicology* 3 (1): 4–6.
- Kawo, A.H., Z.A. Suleiman, and M. Yusha'u. 2011. Studies on the antibacterial activity and chemical constituents of *Khaya senegalensis* and *Ximenia americana* leaf extracts. *African Journal of Microbiology Research* 5 (26): 4562–4568.
- Kelly, R.F., S.M. Hamman, K.L. Morgan, E.F. Nkongho, V.N. Ngwa, V. Tanya, W.N. Andu, M. Sander, L. Ndip, I.G. Handel, S. Mazeri, A. Muwonge, and B.M.D.C. Bronsvort. 2016. Knowledge of bovine tuberculosis, cattle husbandry and dairy practices amongst pastoralists and small-scale dairy farmers in

- Cameroon. *PLoS One* 11 (1): e0146538. <https://doi.org/10.1371/journal.pone.0146538>.
- Kilani, S., M. Ben Sghaier, I. Limem, I. Bouhlej, J. Boubaker, W. Bhouiri, I. Skandrani, A. Neffatti, R. Ben Ammar, M.G. Dijoux-Franca, K. Ghedira, and L. Chekir-Ghedira. 2008. *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. *Bioresource Technology* 99 (18): 9004–9008. <https://doi.org/10.1016/j.biortech.2008.04.066>.
- Kpadonou-Kpoviessi, D.S., S. Kpoviessi, E. Yayi-Ladekan, F. Gbaguidi, B. Yehouenou, M. Mansourou, and G.C. Accrombessi. 2013. Phytochemical screening, antimicrobial activities and toxicity against *Artemia salina* Leach of extracts and fractions of *Ocimum gratissimum* Linn from Benin. *Journal of Chemical and Pharmaceutical Research* 5 (10): 369–376.
- Lobanovska, M., and G. Pilla. 2017. Penicillin's discovery and antibiotic resistance. *Yale Journal of Biology and Medicine* 90: 135–145.
- Mamman, V., B. Mahmud, M.G. Magaji, and N.M. Danjuma. 2019. Sub-acute toxicological evaluation of methanol whole plant extract of *Tapinanthus dodoneifolius* (D.C) Danser in Wistar rats. *Journal of Pharmaceutical Development and Industrial Pharmacy* 1 (3): 16–27.
- McElwain, T.F., and S.M. Thumbi. 2017. Animal pathogens and their impact on animal health, the economy, food security, food safety and public health. *Revue scientifique et technique de l' Office International des Epizooties* 36 (2): 423–433. <https://doi.org/10.20506/rst.36.2.2663>.
- MINEPIA. 2009. Master plan for the development of the livestock sectors in Cameroon. In *Mapping of spinneret Volume II: Mapping of spinneret*, 82. Available at: <https://docplayer.fr/20931055-Schema-directeur-pour-le-developpement-des-filieres-de-l-elevage-au-cameroun.html>. Accessed 28 Feb 2022.
- MINEPIA. 2019 unpublished. Statistical yearbook of the Ministry of Livestock, Fisheries and Animal Industries of Cameroon. 2018.
- Mohamed, E.A.A., A.M. Muddathir, and M.A. Osman. 2020a. Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two *Pulicaria* spp. growing in Sudan. *Scientific Reports* 10 (1): 1–8. <https://doi.org/10.1038/s41598-020-74262-y>.
- Mohamed, M.S., M.T. Idriss, N.K. Alruwaili, N.H. Alotaibi, T. Elsaman, and M.A. Mohamed. 2020b. Investigation of the potential anti-influenza activity of five plants grown in Sudan and used folklorically for respiratory infections. *Journal of Applied Pharmaceutical Science* 10 (06): 054–061. <https://doi.org/10.7324/JAPS.2020.10608>.
- Mostafa, A.A., A.A. Al-Askar, K.S. Almaary, T.M. Dawoud, E.N. Sholkamy, and M.M. Bakri. 2018. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences* 25 (2): 361–366. <https://doi.org/10.1016/j.sjbs.2017.02.004>.
- Motta, P., T. Porphyre, S.M. Hamman, K.L. Morgan, V.N. Ngwa, V.N. Tanya, E. Raizman, I.G. Handel, and B.M. Bronsvort. 2018. Cattle transhumance and agropastoral nomadic herding practices in Central Cameroon. *BMC veterinary research* 14 (1): 1–12. <https://doi.org/10.1186/s12917-018-1515-z>.
- Mouiche, M.M.M., M. Mazra, F. Moffo, S.E. Mpouam, A.L. Ngoujegni, K.J.M. Feussom, et al. 2018. Marché des médicaments vétérinaires au Cameroun: Réglementation, Organisation et Circuits de Distribution. *Bulletin of Animal Health and Production in Africa* 66: 591–605.
- Mthi, S., J.M. Rust, T.L. Morgenthal, and B. Moyo. 2018. An ethno-veterinary survey of medicinal plants used to treat bacterial diseases of livestock in three geographical areas of the Eastern Cape Province, South Africa. *Journal of Medicinal Plants Research* 12 (18): 240–247. <https://doi.org/10.5897/JMPR2017.6444>.
- Mutee, A.F., S.M. Salhimi, F.C. Ghazali, F.M. Al-Hassan, C.P. Lim, K. Ibrahim, and M.Z. Asmawi. 2012. Apoptosis induced in human breast cancer cell line by *Acanthosper planci* starfish extract compared to tamoxifen. *African Journal of Pharmacy and Pharmacology* 6 (3): 129–134. <https://doi.org/10.5897/AJPP11.208>.
- Ncube, N.S., A.J. Afolayan, and A.I. Okoh. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology* 7 (12): 1797–1806. <https://doi.org/10.5897/AJB07.613>.
- Ndamitso, M.M., M. Musah, Z. Mohammed-Hadi, S. Idris, O.J. Tijani, E.Y. Shaba, and A. Umar. 2013. Analysis of phytochemical content and antibacterial activity of *Tapinanthus dodoneifolius* extracts. *Researcher* 5 (5): 54–59.
- Nordin, M.L., A.A. Othman, A.A. Kadir, R. Shaari, A.Y. Osman, and M. Mohamed. 2019. Antibacterial and cytotoxic activities of the *Syzygium polyanthum* leaf extract from Malaysia. *Veterinary World* 12 (2): 236–242. <https://doi.org/10.14202/vetworld.2019.236-242>.
- Pan, Li, L.B.S. Kardono, C.M. Pannell, S. Riswan, D.D. Soejarto, H. Chai, T.G. McCloud, E.J. Carcache de Blanco, D.J. Newman, and A.D. Kinghorn. 2010. Isolation and characterization of minor analogues of silvestrol and other constituents from a large-scale re-collection of *Aglaia foelata*. *Journal of Natural Products* 73 (11): 1873–1878. <https://doi.org/10.1021/np100503q>.
- Prashant, T., K. Bimlesh, K. Mandeep, K. Gurpreet, and K. Harleen. 2011. Phytochemical screening and extraction: a review. *International Pharmaceutica Scientia* 1 (1): 98–106.
- Rortana, C., H. Nguyen-Viet, S. Tum, F. Unger, S. Boqvist, S. Dang-Xuan, S. Koam, D. Grace, K. Osbjør, T. Heng, and S. Sarim. 2021. Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in chicken meat and pork from Cambodian Markets. *Pathogens* 10 (5): 556.
- Skehan, J.R., T.C.P. Dinis, A.P. Cunha, and L.M. Almeida. 1997. Antioxidant activities of some extracts of *Thymus zygis*. *Free Radical Research* 26 (5): 469–478. <https://doi.org/10.3109/10715769709084484>.
- Suter M, C. and Richter. 2000. Anti and pro-oxidative properties of PADMA 28, a Tibetan herbal formulation. *Redox Report* 5:17–22, 1, <https://doi.org/10.1179/1071576005.1.17>.
- Talla, E., T.A. Ngege, P. Biyanzi, P. Sakava, A.P. Forche, M.J. Tanyi, T.N.F. Fohouo, and R. Ndjouenkeu. 2014. Phytochemical screening, antioxidant activity, total polyphenols and flavonoids content of different extracts of propolis from Tekel (Ngaoundal, Adamawa region, Cameroon). *The Journal of Phytopharmacology* 3 (5): 321–329.
- Tanamatayarat, P., P. Limtrakul, S. Chunsakaow, and C. Duangrat. 2003. Screening of some Rubiaceae plants for cytotoxic activity against cervix carcinoma (KB-3-1) Cell Line. *Thai Journal of Pharmaceutical Sciences* 27: 167–172.
- Tavassoli, S., and Z.E. Djomeh. 2011. Total phenols, antioxidant potential and antimicrobial activity of methanol extract of rosemary (*Rosmarinus officinalis* L.). *Global Veterinaria* 7 (4): 337–341.
- Tepe, B., D. Daferera, A. Sokmen, M. Sokmen, and M. Polissiou. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry* 90 (3): 333–340. <https://doi.org/10.1016/j.foodchem.2003.09.013>.
- Varona, O.M., K. Chaintarli, B. Muller-Pebody, M.F. Anjum, T. Eckmanns, M. Norström, et al. 2020. Monitoring antimicrobial resistance and drug usage in the human and livestock sector and foodborne antimicrobial resistance in six European countries. *Infection and drug resistance* 13: 957–993. <https://doi.org/10.2147/IDR.S237038>.
- Vougat Ngom, R.R.B., T. Tomdieu, R. Ziébé, H.S. Foyet, M. Moritz, L. Vondou, D.E. Schrunk, P.M. Imerman, W.K. Rumbelha, and R.B. Garabed. 2017. Quality of veterinary pharmaceuticals and their use by pastoralists in the Far North Region of Cameroon. *Pastoralism: Research, Policy and Practice* 7 (6): 1–14.15.
- Vougat, R.R.B.N., H.S. Foyet, R. Ziebe, and B.R. Garabed. 2015. Antioxidant activity and phytochemical constituent of two plants used to manage foot and mouth disease in the Far North Region of Cameroon. *Journal of Intercultural Ethnopharmacology* 4 (1): 40–46. <https://doi.org/10.5455/jice.20141020064838>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.