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Chemical characterization and biological activities of extracts from two plants (*Cissus quadrangularis* and *Acacia polyacantha*) used in veterinary medicine in Benin

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Abstract
This work focuses on chemical and biological study of two plants, *Cissus quadrangularis* and *Acacia polyacantha* used by farmers in Benin in the treatment of salmonellosis in poultry and cattle gastrointestinal diseases from. The results, we note in both plants the presence of several secondary metabolites such as saponins, catechic tannins, mucilages, flavonoids, anthocyanins, reducing compounds, sterols and terpenes. Regarding the polyphenol compounds content, that of *Acacia polyacantha* is higher than the ones of *Cissus quadrangularis*. As for the test results of the anti-radical activity, extracts from the trunk bark of Acacia polyacantha show better activity compared to those of *C. quadrangularis*. The results of antibacterial activity of all (ethanol, hydroethanolic and aqueous) extracts of *C. quadrangularis* have proven bactericidal towards *S. typhi*. The ethanolic and aqueous extracts are bactericidal at the concentration of 50 mg / ml while the hydroethanolic extract is bactericidal at 0.78 mg / ml with *S. typhi*.

Keywords: *Cissus quadrangularis*, *Acacia polyacantha*, secondary metabolites, antiradical, bactericidal

1. Introduction
Cissus quadrangularis (syn*: Vitis quadrangularis*) is a herbaceous plant belonging to the family of Vitaceae, reaching a height of 1.5 m [1]. Its leaves are simple or lobed, cordate, broadly ovate or kidney-shaped serrated. These flowers are small, greenish, bisexual, tetramers. These fruits are fleshy berries obovoid [1, 2]. *C. quadrangularis* grows in hot, dry areas. Its stem is branched, glabrous, fibrous and smooth [3].
Acacia polyacantha (syn: *Acacia campylacantha*), belonging to the family of Mimosoideae is a thorny tree ranging from 16 m to 25 m hight [4, 5]. It is a flat and spreading crown to tree bark gray, yellow or blackish, fissured and scaly [5].
In Benin, livestock is one of the first activities undertaken by the man after agriculture to ensure food security and survival. Salmonellosis and gastrointestinal infections are the major cause of mortality and economic losses in this sector. The very attached to their poultry and their livestock, farmers can't remain indifferent to the health of these and use the active principles of plants to fight against certain diseases which affect them [6]. This behavior of farmers is often favored by the affordable cost of herbal medicines and the resistance of some pests to synthetic products [7].
Cissus quadrangularis and *Acacia polyacantha* are part of the plants used by farmers to treat diseases of livestock, such as Salmonellosis and gastrointestinal diseases in general. These treatments are undervalued because the tests that confirm their effectiveness are still very few and poorly understood. To correct this failure, this work aims chemical and biological studies of these two plants. Specifically, it is to identify secondary metabolites, determine the levels of polyphenolic compounds, evaluate anti-radical and antibacterial activities of the extracts of *Cissus quadrangularis* and *Acacia polyacantha*

2. Materials and methods
2.1. Materials
2.1.1. Plants material
The plant material used in this study was made from the trunk bark of *Acacia polyacantha* and *Cissus quadrangularis* harvested in January 2014 in Dassa, southern areas of Benin.

2.1.2. Animal material
It consisted of the reference strains of *Staphylococcus aureus* (ATCC 27844), *Escherichia coli* (O:157H7), *Salmonella typhi* (R.0951401) and *Klebsiella pneumoniae* (ATCC 35657). These strains were provided by the National Health Laboratory of Benin (Ministry of Health).

2.2. Methods
After collecting over the plant material, the samples were dried over laboratory temperature (25-30 °C) until their stabilization and then reduced in powder.

2.2.1. Identification of secondary metabolites
Determination of secondary metabolites was made by staining reactions and precipitation specific to each metabolite family.

Flavonoids: Flavonoids identification was carried out by the test of cyaniding [8].

Tannins: They have been highlighted by the Stiasny test [9].

Saponins: The saponins were determined by foam test; degree of aqueous decoction dilution giving a persistent foam after shaking [10].

Polyphenols: Identification of compounds belonging to the group of polyphenols was made by the reaction with ferric chloride [8].

Terpens and sterols: Sterols and terpens have been identified by the Liebermann-Burchard test [11]

Alkaloids: Alkaloids were identified by Meyer test and confirmed by Bouchardat test [12, 13]
Anthraquinone: They were identified by Bornträger test [10].

Mucilages: Obtaining a decoction of a flocculent precipitate in ethyl ether indicated the presence of mucilages [14].

Coumarins: Coumarins were identified by UV fluorescence at 365 nm[9] .

Volatile compounds: The volatile compounds were identified by the hydro distillation method using an extractor of Clevenger type [15, 16].

2.2.2. Preparation of extracts
The technique used was that of maceration. 50 g of each powder sample were introduced into a 500 ml flask containing 250 ml of extraction solvent (ethanol, water or ethanol water (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. The yield (Y) of extraction was calculated by the formula below. Y (%) = (Mass of extract)/ (Mass of plant material used) X100.

2.2.3. Determination of polyphenolic compounds
Total polyphenols: The total phenolic content of the various extracts was quantified using the Folin–Ciocalteu reagent according to Singleton *et al* [17, 18]. This method consisted to use a mixture of phosphotungstic and phosphomolybdic acids, which were reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum [19]. The absorbance was measured by a spectrophotometer (JENWAY 50/60 Hz) to 765 nm. Gallic acid was used as reference and the total polyphenol 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 technique was based on the formation of the aluminum complex flavonoids that had a maximum absorption at 500 nm [20, 21].

Condensed tannins: The condensed tannins dosing was achieved by the method of sulfuric vanillin [22]. 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 red colored complex chromophore which absorbed at 510 nm.

2.2.4. Evaluation of scavenging activity
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 purple color of DPPH. The mixture of DPPH solution and the sample was left in the darkness for an hour and the absorbance measured at 517 nm [23, 24]. The trapping percentage was determined by the formula: P=(AbW-AbS)/Ab) X100; P: percentage of trapping; AbW: absorbance of the white; AbS: Absorbance of the sample.

2.2.5. Determination of antibacterial activity
The antibacterial activity was evaluated in microplates and in Petri dishes according to the literature [25, 26, 27].

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

3. Results and discussion
3.1. Secondary metabolites
Secondary metabolites identified in the stem bark of *A. polyacantha* and *C. quadrangular*
In the stem bark of *A. polyacantha* Dassa (Benin), the saponins, catechic tannins, alkaloids, anthocyans, leuco-anthocyanin, coumarins, sterols and terpenes were highlighted. At the trunk bark of the same plant harvested in Zaria (Nigeria), Okpanachi *et al.* [28] noted the presence of flavonoids and anthraquinones which were absent in the sample of Benin. As regards the *C. quadrangularis* Dassa (Benin), it contains polyphenols, catechic tannins, flavonoids, reducing compounds, sterols and terpenes as a work of **Srivastava *et al*** [29] demonstrated in India contains proteins, mucilages and quinones which have not been identified in *C. quadrangularis* of Benin.
The change in metabolites noticed in our samples from previous work might be related to the period from harvest, to the soil or climate factors [30, 31]. Diversity secondary metabolites of these species could explain their uses in the treatment of inflammatory diseases; diarrhea; parasitic, salmonellosis and gastrointestinal diseases. The presence, such as tannins and flavonoids in these plants would justify their use in the treatment of diarrheal diseases and gastrointestinal diseases of animals [32]

Table 1: Metabolites identified in the trunk bark of A. polyacantha and C. quadrangularis

Secondary metabolites		*A. polyacantha*	*C. quadrangularis*
Alkaloids		+	-
Flavonoids		-	+
Anthocyanins		-	-
Leuco-anthocyanins		+	-
Anthraquinones		-	-
Free anthraquinones		-	-
Combined anthraquinones	O-heterosides	-	-
	O-heteroside with reduced genine	-	-
	C-heterosides	-	-
Reducing Compounds		-	+
Tannins	Gallic	-	-
	Catechic	+	+
Sterols and terpenes		+	+
Mucilages		-	-
Saponosides		+	-
Coumarins		+	-
Quinones		+	-
Proteins		-	-
Polyphenols		-	+
volatile compounds		-	-

+: presence;-: absence

3.2. Extraction yields

Extraction yields of the stem bark of *A. polyacantha* and *C. quadrangularis* were shown in Table 2.

In this table the yields of ethanolic, hydroethanolic and aqueous extracts were respectively 5.6%; 15.2% and 9.2% for *A. polyacantha* and 6.8%; 15.6% and 14.0% with regard to *C. quadrangularis*. The ethanol-water binary (50/50) allowed having a high efficiency in both plants.

Table 2: Extraction yields of *A. polyacantha* and *C. quadrangularis*

	Yieds (%)	
Extracts	***A. polyacantha***	***C. quadrangularis***
Ethanolic	5.6	6.8
hydroethanolic	15.2	15.6
Aqueous	9.2	14.0

3.3. Polyphenolic compounds contents

The total polyphenols content, total flavonoids and condensed tannins of the extracts A. polyacantha and C. quadrangularis were displayed in Table 3.

Table 3: Polyphenolic compounds contents of the extracts of *A. polyacantha* and *C. quadrangularis*

Polyphenolic compounds contents	Extracts	*A. polyacantha*	*C. quadrangularis*
Total polyphenol (mg GAE/g DM)	Ethanolic	5.547±0.705	3.384±0.044
	hydroethanolic	6.768±0.018	4.768±0.132
	Aqueous	4.730±0.908	1.845±0.071
Total flavonoids (mg CE/g DM)	Ethanolic	18.659±0.023	10.210±0.639
	hydroethanolic	22.698±3.541	10.291±0.206
	Aqueous	19.418±0.548	5.977±0.183
Condensed tannins (mg CE/g DM)	Ethanolic	52.093±0.789	10.791±1.052
	hydroethanolic	30.047±1.973	10.605±0.789
	Aqueous	17.302±0.526	7.442±0.263

In Table 3, the total polyphenol content of ethanolic, hydroethanolic and aqueous extracts of *A. polyacantha* were respectively (5.547±0.115) mg EAG/gDM, (6.769±0.018) mgEAG/DM and (4.730±0.908) mgEAG/gDM. As for the total flavonoids content extracts of the same plant, the ethanolic extract of the was (18.659±0.023) mgCE/gDM while the content of the hydroethanolic extract was (22.698±3.541) mg CE/gDM and a content of (19.418±0.548) mg CE /gDM for the aqueous extract. However, levels of condensed tannins of ethanolic, hydroethanolic and aqueous extracts were respectively (52.093±0.789) mg CE/gDM, (30.047±1.973) mg CE/g DM and (17.302±0.526) mg CE/g DM. Tukey's test (ANOVA) showed that the extraction solvent did not significantly influence the total polyphenol contents and total flavonoids but influenced the condensed tannins in *A. polyacantha* ($P < 0.05$). As for *C. quadrangularis*, the polyphenol content of the ethanolic extract was (3.384±0.044) mg EAG /g DM while that of the hydroethanolic extract gave a content of (4.768±0.132) mg EAG / g DM with a low content of the aqueous extract (1.845±0.070) mg EAG/gDM. Regarding the total flavonoids content, the ethanol extract of the (10.210±0.639) mg CE/ gDM and the hydroethanolic extract was (14.621±0.206) mg CE / g DM with a low at the level of the aqueous extract (5.977±0.183) mg CE/g DM. As for the condensed tannins contents of ethanolic, hydroethanolic and aqueous extracts stem bark of *C. quadrangularis*, they were respectively (10.791±1.052) mg EC/gDM, (10.605±0.789) mg CE/gDM and (7.442±0.263) mg CE /g DM. Statistically, it was noted that according to Tukey test the extractant significantly influenced the content of polyphenolic compounds in *C. quadrangularis*.

3.4. Radical scavenging activity of the extracts from the trunk barks of *C. quadrangularis* and *A. polyacantha*

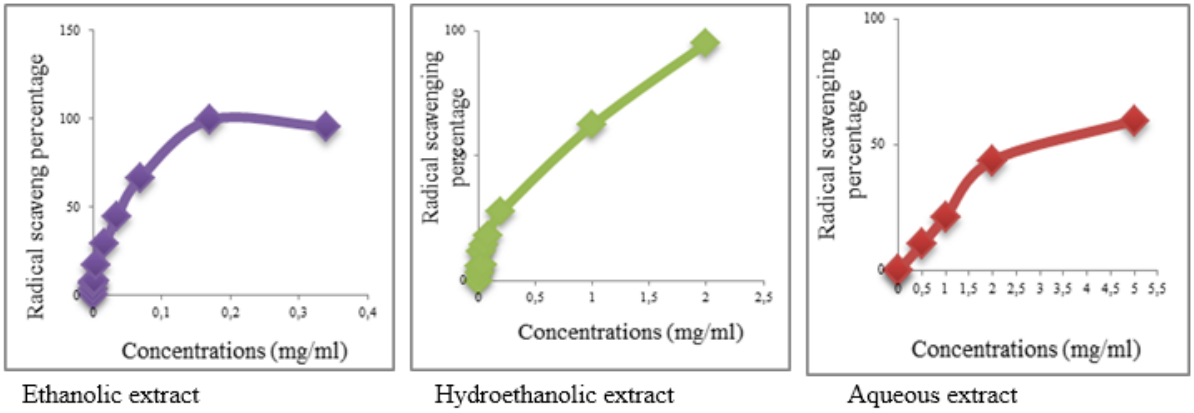

Fig 1: Antiradical activity of stem of the extracts of *C. quadrangularis*

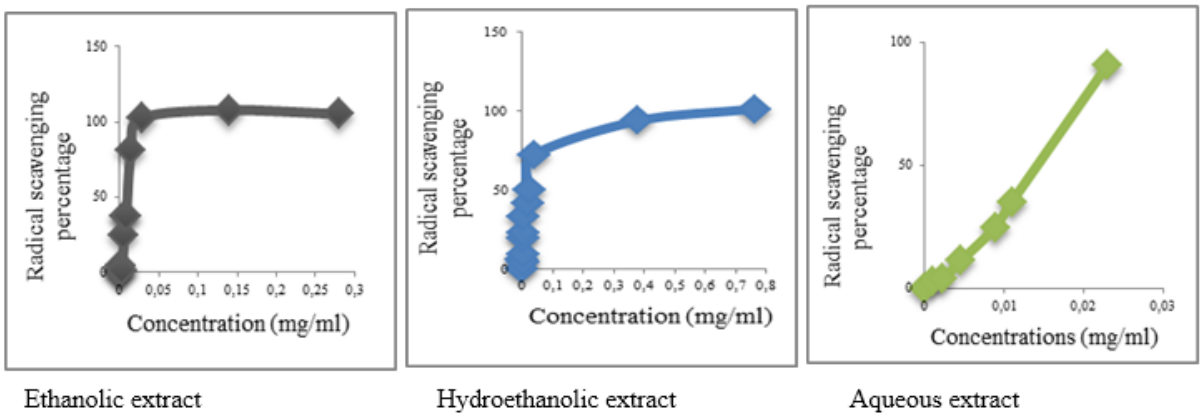

Fig 2: Antiradical activity of stem bark of the extracts of *A. polyacantha*

Figures 1 and 2 showed the percentage of free radical scavenging versus concentrations of the extracts of *C. quadrangularis* and *A. polyacantha*

For these three extracts (ethanolic, hydroethanolic and aqueous) there was a progressive increase in trapping a percentage depending on the concentration of the extracts of *C. quadrangularis*. Concentrations for trapping free radicals 50% (IC_{50}) of the ethanolic, Hydroethanolic and aqueous extracts of C. quadrangularis were respectively 40 µg/ml, 660 µg/ml and 3000 µg/ml. After analyzing the results, we noted that the increase in the extraction solvent polarity decreased the antiradical activity of the extracts of the stem of *C. quadrangularis*. The aqueous extract of *C. quadrangularis* was less rich in polyphenolics (total polyphenols, flavonoids and tannins) than the ethanolic and hydroethanolic extracts and had negligible scavenging activity compared with the latter. One could conclude that the antiradical activity of the extracts of *C. quadrangularis* was related to their content of polyphenolic compounds, which was in accordance with previous work [33]. As to the extracts of *A. polyacantha*, the percentage of trapping, increased with the concentration of the extracts. From the figure the IC_{50} of ethanolic, hydroethanolic and aqueous extracts were respectively 10 µg/ml, 20. µg/ml and 14 µg/ ml. The ethanolic extract showed a higher scavenging activity than the hydroethanolic and aqueous extracts. The extracts of *A. polyacantha* were more active than those of *C. quadrangularis.* All samples of these two plants were less active than BHA (IC_{50}=4, 8 µg/ml) which was a synthesis of antiradical.

3.5. Antibacterial activity

3.5.1. *Extracts of A. polyacantha*

The table 4 indicated the Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) and antibiotic power of the extracts of *A. Polyacantha.*

The ethanolic and hydroethanolic extracts of *A. polyacantha* showed bactericidal activity with *S. aureus* a strain which was resistant to the aqueous extract of the same plant. Regarding *E. coli*, the ethanolic extract showed a bacteriostatic activity while the hydroethanolic extract inhibits this strain. As for *K. pneumoniae*, the three extracts (ethanolic, hydroethanolic and aqueous) were inactive with displayed to this strain. The ethanolic and aqueous extracts of the stem bark of *A. polyacantha* displayed bacteriostatic activity with *S. typhi* while the hydroethanolic extract inhibited this strain at a concentration of 50 mg/ml.

3.5.2. Extracts of *C. quadrangularis*

The Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) and antibiotic power of the extracts of *C. quadrangularis* were shown in Table 5.

Table 4: MIC, MBC and antibiotic power of the extracts of *A. polyacantha*

Microorganism Strains	Extracts	Concentrations (mg/ml)		CMB/CMI
		CMI	***CMB***	
S. typhi	Ethanolic	6.25	50.00	8.00
	hydroethanolic	50.00	>100.00	>2.00
	Aqueous	12.50	100.00	8.00
S. aureus	Ethanolic	1.56	6.25	4.00
	hydroethanolic	25.00	50.00	2.00
	Aqueous	>100.00	>100.00	>1.00
E. coli	Ethanolic	3.13	25.00	8.00
	hydroethanolic	50.00	>100.00	>2.00
	Aqueous	>100.00	>100.00	>1.00
K. pneumoniae	Ethanolic	>100.00	>100.00	>1.00
	hydroethanolic	>100.00	>100.00	>1.00
	Aqueous	>100.00	>100.00	>1.00

Table 5: MIC, MBC and antibiotic power of the extracts from *C. quadrangularis*

Microorganism strains	Extracts	Concentrations (mg/ml)		CMB/CMI
		CMI	CMB	
S. typhi	Ethanolic	25.00	50.00	2.00
	hydroethanolic	0.39	0.78	2.00
	Aqueous	12.50	50.00	4.00
S. aureus	Ethanolic	25.00	>100.00	>4.00
	hydroethanolic	25.00	>100.00	>4.00
	Aqueous	6.25	50.00	8.00
E. coli	Ethanolic	6.25	50.00	8.00
	hydroethanolic	1.56	50.00	32.05
	Aqueous	100.00	>100.00	>1.00
K. pneumoniae	Ethanolic	>100.00	>100.00	>1.00
	hydroethanolic	>100.00	>100.00	>1.00
	Aqueous	>100.00	>100.00	>1.00

The three extracts (ethanolic, hydroethanolic and aqueous) *C. quadrangularis* showed bactericidal activity towards the strain of *S. typhi*. For *S. aureus*, the aqueous extract shows a bacteriostatic activity with this strain at a concentration of 50 mg/ml while the ethanolic and hydroethanolic extracts inhibited it only. As for the strain of *K. pneumoniae*, it was insensitive to the extracts of *C. quadrangularis.* The ethanolic and hydroethanolic extracts showed bacteriostatic activity towards *E. coli*, but there was an inhibitory activity of the aqueous extract with it.

4. Conclusion

The present work aims to chemical and biological studies of two plants, *C. quadrangularis* and *A. polyacantha* were used by farmers in Benin in the treatment of salmonellosis in poultry and livestock gastrointestinal diseases.

From the results, it appears that these two plants are rich in secondary metabolites and the ethanol-water binary allows for a better extraction efficiency of these metabolites from these two plants. On level of polyphenolic compounds, *A. polyacantha* extracts are richer than those of *C. quadrangularis* on. From this point of view of the radical-scavenging activity, extracts of *A. polyacantha* have a stronger activity than *C. quadrangularis*. Extracts of *C. quadrangularis* show bactericidal activity towards *S. typhi*. The diversity of secondary metabolites and biological activities noted in extracts could justify the use of these plants by farmers to treat livestock and poultry diseases like salmonellosis.

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