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Identification and quantification of phenolic compounds and flavonoids in anthelmintic ethnoveterinary plants used among Fulani and Mosse, Central Burkina Faso

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Abstract

Helminthic diseases are the leading cause of loss of animals in the Sahel region of Western Africa. Poverty, combined with the poor development of modern veterinary services, forces small farmers to use herbals to treat their livestock. A previous ethnoveterinary survey, carried out in the region of Saba in Burkina Faso, indicates a frequent use of the leaves of *Acacia macrostachya* DC. (Leguminosae), *Combretum glutinosum* Perr. ex DC. (Combretaceae) and *Mitragyna inermis* (Willd.) Kuntze, to treat helminthic diseases.

Phytochemical screening has allowed us to characterize sterols, triterpens, flavonoids and tannins as the main phytochemical classesof these 3 species; phenolic compounds range from 2.5 to 8 % and a series of bioactive polyphenols (quercetin, rutin, gallic acid) have been identified by thin-layer chromatography. The obtained results indicate the richness of these herbs in phenolic compounds. The biological activity, toxicology and phytochemistry of the 3 species warrant further investigation to justify their ethnoveterinary uses.

Keywords: herbalanthelminthics, polyphenols, quercetin, *Acacia macrostachya, Combretum glutinosum, Mitragyna inermis.*

Introduction

In Western Africa, livestock plays an important economic role, representing, for example,in Burkina Faso 80% of the rural population incomes and 26% of export earnings[1] (INSD, 2007). Unfortunately, the quality of livestock performance has remained poor as a result of a number of animal diseases (particularly intestinal parasitoses and trypanosomiasis). The treatment of gastrointestinal helminthiasis is particularly difficult because of polyparasitism and frequent re-infestations [2]. Moreover different studies highlight the worldwide emergence of phenomena of resistance to the existing anthelmintics[3,4,5] In rural regions of Burkina Faso, helminthiasis remains a major concern because of a poor veterinarian coverage complicated by the high cost of modern drugs; as a consequence, the majority of livestock breeders chiefly rely on ethnoveterinary practices and herbal medicines.

Despite this importance of ethnoveterinary medicine for African agriculture, there still remains a lack of laboratory work to validate the use of herbals, to elucidate their active compounds and mechanisms of action and to precise their advantages, limitations and precautions of use [6,7].

A recent ethnoveterinary survey we carried out in the Saaba region in Burkina Faso indicated that the leaves of some species [*Acacia macrostachya* DC. (Leguminosae), *Combretum glutinosum* Perr.ex DC. (Combretaceae) and *Mitragyna inermis* (Willd.) Kuntze] are frequently used by breeders to treat helminthic diseases.

Acacia macrostachya is ascandent shrub whith creamy-white flowers on a pubescent peduncle. The leaves andyoung shoots areused to treat gastrointestinal disorders (diarrhea, nausea), as an anthelminticand as an antidote to snake bites [8, 9]. *Combretum glutinosum* is a tree up to 12 m high, recognizable by its large leaves. The bark is used for the treatment of influenza and rheumatism. The roots possess anthelmintic properties, treat cough,stomach problemsand gonorrhea.Young shoots, alone or in combination withthe bark, have aphrodisiac properties and treat bleeding and gonorrhea [10,11] *Mitragyna inermis* is a shrub or tree, dense and spherical in appearance, up to 16 m high, with highly fragrant globe-shaped flowers (2 to 3.5 cm in diameter).The bark is commonly used against bloating, diarrhea, dysentery and disorders of childbirth [10,11].

No information has been published so far on possible anthelminthic phytochemicals from these 3 plants; the present study aims at screening their major phytochemical classes and at quantifying their total phenolic and flavonoid compounds.

Materials and Methods

Stems with leaves of the three species (*Acacia macrostachya*, *Combretum glutinosum,* and *Mitragyna inermis*) were collected at Saaba, identified by J. Millogo, botanist (University of Ouagadougou), dried in the shade at room temperature and powdered. Voucher herbarium specimens were deposited in the University herbarium ("Rappez" serie).

Extraction

The plant powder (25 g) was extracted using a Soxhlet system with 250 ml chloroform and then 250 ml methanol (until clear supernatant; 3 to 8 h cycling). The extracts were then dried using a Rotavapor system.

Phytochemical screening

The phytochemical screening consisted of a qualitative analysis of secondary metabolites present in the plant samples using classical color or precipitation reactions. Reactions were carried out (i) in chloroform extracts, for sterols, triterpens, flavonoid and coumarin aglycones were researched; and (ii) in methanol extracts, for flavonoids, catechic and gallic tannins, saponins, anthraquinones, alkaloids and coumarins.

Thin-layer chromatography profiling of polyphenols

Two ml of aqueous methanol solution (10 mg of methanol extract / ml) were extracted twice with 5 ml of ethyl acetate; the ethyl acetate extract was evaporated to dryness,dissolved in methanol and applied (10 µl) on silica gel plates (FLUKA, TLC-PET foils). The plate (5cmx 10 cm) was eluted over 8.5 cm with ethyl acetate - n-hexane - acetic acid - water [8 : 2 : 2 : 0.4 (Figure 1) and 10 : 2 : 2 : 0.4 (Figure 2)], dried,treated with a 10 g/L solution of diphenylboric acid aminoethyl ester in methanol and then with a 50 g/L solution of macrogol 400 in methanol and examined in ultraviolet light at 365 nm.

Determination of total polyphenols, flavonoids and tannins.

Theses determinations were performed on methanol extracts (10 mg/ml).

Total polyphenols were estimated by measuring the reduction of a phosphomolybdotungstic reagent (Folin-Ciocalteu reagent), expressing the concentrations in mg gallic acid equivalents per 100 mg of extract (mg GA/100 mg) [12].

Flavonoids were determined by the method of Dowd adapted by Arvouet-Grand[13], based on the colorimetry of aluminium complexes. The results are expressed in mg quercetin equivalents per 100 mg of extract (mg EQ/100 mg).

Tannins [13] were measured by colorimetry of ferric complexes, calibrating with a tannic acid solution. Results are expressed as mg of tannic acid equivalents per 100 mg of extract (mg TA/100 mg).

Results

Extraction yields

Generally, methanol allows to recover more substances than chloroform, as indicated by a higher extraction yield. This is the case for *Combretum glutinosum* and *Mitragyna inermis* samples (Table I); by contrast the yield of the *Acacia macrostachya* chloroform extraction is substantial, equivalent to the methanolic extract. As phenolic compounds are generally more polar or exist in form of heterosides, the methanol extracts were used for bothpolyphenols TLC identification and spectrophotometric assays.

Table 1 – extraction yield.

	chloroform extract	methanol extract
Acacia macrostachya	16.8 %	16.9 %
Combretum glutinosum	6.7 %	28.0 %
Mitragyna inermis	11.3 %	30.8 %

Table 2:–Phytochemical screening results.

	Acacia macrostachya		*Combretum glutinosum*		*Mitragyna inermis*	
	Chlor. ex.	Meth. ex.	Chlor. ex.	Meth. ex..	Chlor. ex.	Meth. ex.
Sterols / triterpens	+	-	+		+	-
Aglycones and flavonoids	-	+	+	+	-	+
Gallic/ catechic tannins	-	+	+	+	-	+
Saponines	nt	+	nt	+	nt	-
Anthracenosides	nt	-	nt	+	nt	-
Alkaloids	nt	-	nt	-	nt	-
Coumarins	-	nt	-	nt	-	-

Ex. : extract; chlor. : chloroform; meth. : methanol; nt : not tested; +: positive; -: negative

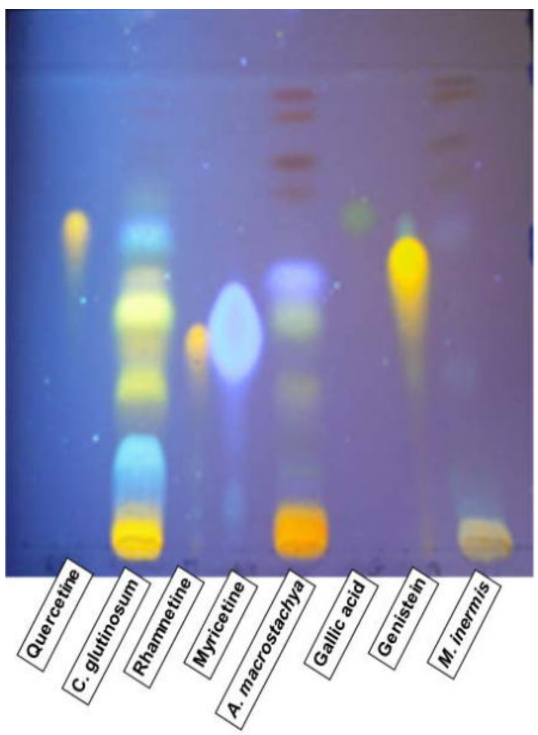

Figure 1: TLC fingerprint profiles ofmethanolic extracts of *Combretum glutinosum*, *Acacia macrostachya* and *Mitragyna inermis*stems with leaves under 366 nm after spray with2-aminoethyl **diphenylborinate**/PEG 2000reagent.
Mobile phase: ethyl acetate - n-hexane - acetic acid - water (8 : 2 : 2 : 0.4)

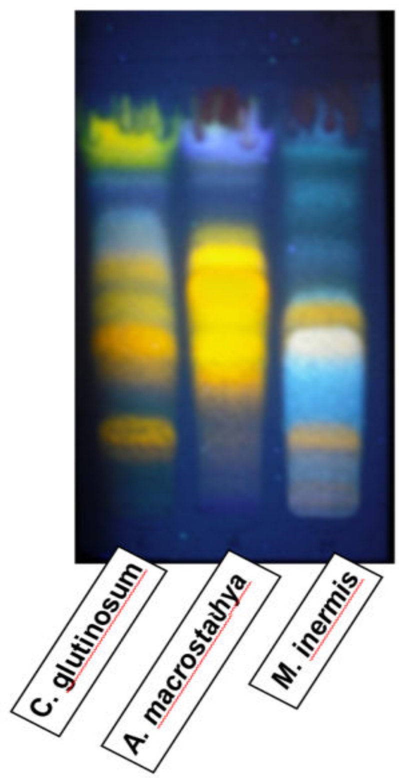

Figure 2: TLC fingerprint profiles ofmethanolic extracts of *Combretum glutinosum*, *Acacia macrostachya* and *Mitragyna inermis*stems with leaves under 366 nm after spray with2-aminoethyl **diphenylborinate**/PEG 2000reagent.
Mobile phase: ethyl acetate - n-hexane - acetic acid - water (10 : 2 : 2 : 0.4)

Phytochemical screening

Acacia macrostachya leafy stems were found to contain triterpenoids, including sterols and saponins, flavonoids and tannins (Table II). In the extracts of *Combretum glutinosum*, triterpenoids, including sterols and saponins, flavonoids, tannins and anthracenoids were detected; previous investigations also indicated the presence of tannins, flavonoids and saponins [10] .The screening of *Mitragyna inermis* extracts highlighted triterpenoids, including sterols, flavonoids and tannins.

Alkaloids previously mentioned in the literature in *Acacia macrostachya* [10,11] nor in *Mitragyna inermis* (known for indole and oxy-indole alkaloids[11,14,15,16] were not detected here; as no acid-base treatment was included in our extraction scheme, this indicates that eventually present alkaloids may be in close combination with tannins.

TLC profiling

Methanolic extracts were fingerprinted by thin-layer chromatography (TLC) which is a common, rapid and cost-efficient method used for characterizing herbal extracts (Figures 1 and 2). The proposed elution and detection systems allow a clear differenciation between the three herbs and could be a basis for developing a pharmacopoeia-style quality monograph; a more polar eluent may however be desirable to separate the compounds presently at low Rf (Figure 2). So far, to our best knowledge, no flavonoids have been identified in these herbs[10] and so several reference substances were tested (example in Figure 1); bands compatible *(i)* with rutin were detected in the extracts of *C. glutinosum* and *Mitragyna inermis*, and *(ii)* with quercitrin in *C. glutinosum* and *A. macrostachya*. These could be used as pharmacopoeial Rf markers to finely position and describe the bands of interest to identify the herb.

Assays

Regarding polyphenolic compounds, the leafy stems of *Acacia macrostachya*, *Combretum glutinosum* and *Mitragyna inermis* were found to contain, respectively *(i)* 9.9 %, 4.7 % and 8.1% of total polyphenols (expressed as % of gallic acid equivalents); *(ii)* 8.0 %, 2.5 % and 5 % of flavonoids (expressed as % of quercetin equivalents); and *(iii)* 6.0 %, 2.0 % and 4.0 % of tannins (expressed as % of tannic acid equivalents). Although the "total polyphenols" include part of flavonoids and tannins, differences in reactivities towards the chromogenic reagents make it difficult to reconciliate the measured percentages.

This high level of polyphenols, especially flavonoids, in the three herbs represents a good premise for bioactivity.

Discussion

We could not identify in literature any study on the anthelmintic activity of *Acacia macrostachya.* Other species of Acacia, *Acacia oxyphylla* Graham ex. Bentham [17], *Acacia molissima* (probably a synonym of *Acacia decurrens* Willd.) [18] *Acacia auriculiformis* Benth, *Acacia nilotica* (exact botanical name not specified) and *Acacia karoo* (exact botanical name not specified) have however been previously investiigated for this activity.*A. oxyphylla* extracts were tested on *Ascaridia galli* with significant dose-dependent nematicide effects comparable to piperazine, a GABA agonist anthelminthic [19] ; the extract of *A. oxyphylla* caused a rupture of the cuticle and damage to muscle fibers of the parasite [17] . The condensed tannins extracted from *A. molissima*were active on lambs naturally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*, yielding significant reductions in faecal egg count (approximately 92%) and charge of *Haemonchus contortus* in the abomasum (approximately 64%). *A. auriculiformis* was active on *Dirofilariaimmitis* in dogs [20] , on *Setaria cervi* and *Hymenolepis diminuta* [21] . *A. karoo*,but not *A. nilotica*, leaves were active on *Haemonchus contortus*in goats, with a significant decrease in the faecal egg count [22] . Our phytochemical screening of *A. macrostachya* revealed the presence of tannins, and it is possible that these molecules are responsible for the traditional anthelmintic use of this plant. This species is the richest for the 3 types of phenolic compounds here tested. Reported anthelminthic activities for other species of *Acacia* indicate that *A. macrostachya* could have high potential for phytomedecine formulation.The eventual presence of alkaloids remains to be confirmed and their structure identified. Extracts from this species have also shown interesting antioxidant and anticancer activities [23] .

Combretum glutinosum is known for antischistosomal, antibacterial, antifungal, antitussive, antiviral, molluscicidal and abortifacient properties but no study so far has shown anthelmintic properties [24]. Twenty *Combretum* species were investigated *in vitro* for the activity of their aqueous, acetone and ethyl acetate extracts against *Caenorhabditiselegans* var. Bristol [25] .The lowest polarity extracts were the most active nematocides. As many species of the genus *Combretum* have an ethnoveterinary anthelminthic indication, further chemotaxonomy studies may allow discovering the active phytochemicals.

Mitragyna inermis extracts were shown antibacterial and antifungal [14] ; the bark extracts presented muscle relaxant and antispasmodic activities[26] and so far no anthelmintic activity has been published. A recent study indicates that the methanolic extract of *Mitragyna parvifolia* (Roxb.) Korth. stembark is active *in vitro* against earthworms, but at quite high concentration (100 mg/ml) [27].

Conclusion

The present study indicates that *Combretum glutinosum, Mitragyna inermis*and *Acacia macrostachya* are rich in polyphenols and flavonoids. Preliminary phytochemical screening and TLC profiling allowed characterizing the 3 herbs and indicating the probable presence of bioactive compounds, quercitrine, rutin and gallic acid. Given the high presence of tannins, these may participate to the activity of the herbs, but may also impart toxic/antinutritional properties to the drugs, which should be evaluated. The biological activity, toxicology and phytochemistry of the 3 species warrant further investigation to justify their ethnoveterinary uses; *Acacia macrostachya* is a rich source in polyphenols and could be prioritized for further studies.

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Author's contributions

FR, AH and PD have made contributions to conception and design, acquisition of data, analysis and interpretation of data.FR, AH and PD have been involved in drafting the manuscript.

Conflict of interest

We have no conflict of interest to declare.

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