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Research Article

ANTI-BACTERIAL ACTIVITY AND PHYTOCHEMICAL COMPOSITION OF EXTRACTS OF THREE MEDICINAL *ASTERACEAE* SPECIES FROM BURKINA FASO.

SAMSON GUENNE*, ADAMA HILOU, NABERE OUATTARA AND ODILE G. NACOULMA

Laboratoire de Biochimie et de Chimie Appliquées (LA.BIO.C.A), Université de Ouagadougou (U.F.R/S.V.T), Ouagadougou, Burkina Faso, Email: guesams@yahoo.fr

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ABSTRACT

The aim of this study was to investigate anti-bacterial activities and phytochemical composition of three Asteraceae (*C. americanum*, *E. alba* and *V. colorata*) from traditionally medicine of Burkina Faso. Inhibition zone diameters of extracts and minimum inhibitory concentration (MIC) of Gram-negative and Gram-positive strains were determinate using standard methods. Preliminary phytochemical tests and polyphenols contents of extracts were evaluated to explain their anti-bacterial activities.Good diameters of inhibition zones were obtained. *E. alba* butanol fraction presented best MIC (1.25mg/mL for *S. typhimurium* (salad), *P. mirabilis (ATCC: 35659) and S. aureus* (ATCC: 6538); 0.625mg/mL for *V. cholerae* (water) and 0.3125mg/mL for *B. cereus* (ATCC: 9144)) for several strains used and the highest in phenolic content (88.88 ± 0.7GAE/100mg of extract). This phytochemical profile would explain the anti-bacterial activities of extracts of plants. Our study shows that *C. americanum* could be also used for the treatment of some bacterial infection diseases. *C. americanum* and *E. alba* are good ways for Burkina Faso future research to isolate new anti-bacterial compounds.

Keywords: Anti-infectious herbs; *C. americanum*; *E. alba*; *V. colorata*; Phenolics; *Asteraceae*; Burkina Faso

INTRODUCTION

Antibiotic resistance is an increasing global problem. This threatens the successful treatment of bacterial infections of humans and animals, especially in Africa[1]. Indeed, many bacterial strains responsible for infectious diseases become resistant to several antibiotics (ampicillin, ciprofloxacin, gentamicin ...). The results of studies show such a high percentage of nosocomial infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci[2]. Due to the low development of the conventional health system in Africa, over 80% of the population uses the recipes from traditional Medicine[3]. These medicinal plants contain many different bioactive phytochemicals for the treatment of various diseases. An ethnobotanical survey has shown that in the central region of Burkina Faso *Chrysanthellum americanum* (L.) Vatke, *Eclipta alba* (L.) Hassk. and *Vernonia colorata* (Willd.) Drake are among the most traditionally used species to treat infectious diseases[4, 5, 6]. To add value to Burkina Faso flora of and to participate to international initiative to search for new drug molecules from plants, this study aimed to evaluate the antibacterial activities of these three species. The well-known properties of phytochemicals identified in extracts help to explain the activities[7].

MATERIALS AND METHODS

Chemicals

To carry out our activities (phytochemical screening and anti-bacterial activity), we used solvents and various classic reagents. All reagents were analytical grades. Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium hydroxide, gallic acid, quercetin, aluminium trichloride ($AlCl_3$), hydrochloric acid, magnesium chloride, ethyl acetate, p-iodonitrotetrazolium chloride (INT) and n-hexane were purchased from Sigma Aldrich chemie (Steinheim, Germany). Ammonium ferric citrate and potassium persulfate were supplied by Fluka chemie (Buchs, Switzerland). Dichloromethane, sulfuric acid, acetic anhydride, ferric trichloride, chloroform, ethanol and methanol were sourced from Probalo (Paris, France). Butanol was sourced from sds (Peyin, France); ascorbic acid and tannic acid were supplied by labosi (Paris, France).

Plants materials

Chrysanthellum americanum (L.) Vatke, *Eclipta alba* (L.) Hassk. and *Vernonia colorata* (Willd.) Drake (*Asteraceae* species), were collected in August 2008 at Loumlila, 15 Km north of Ouagadougou, capital of Burkina Faso. The plants were identified by Prof. Millogo-Rasolodimby from the plants Biology Department of the University of Ouagadougou. A voucher specimen (ID-10474, ID-10476 and ID-10478 respectively) was deposited at the Herbarium of the Laboratory of Vegetable Biology and Ecology, of the University of Ouagadougou.

Preparation of Extracts

Leaves of *V. colorata* and whole-plants of *C. americanum* and *E. alba* were dried at room temperature and ground to fine powder. Twenty five grams of powdered plant material was extracted with 250ml of aqueous-ethanol (80%) at laboratory conditions during 24 hours. After, extracts solution were concentrated under reduced pressure in a rotary evaporator (BÜCHI, Rotavopor R-200, Switzeland) at approximately 40°C, frozen and lyophilized using a lyophilizer (Telstar-Cryodos 50, Spain). The extracts (crude) obtained were fractionated by solvents of increasing polarity (hexane, dichloromethane, ethyl acetate and butanol). All obtained extracts were weighed before packed in waterproof plastic flasks and stored at 4°C until use.

Antibacterial Study

Microorganisms

Eleven microorganisms used in this study consisted of clinical isolates and collection/ sereotyped strains (Gram positive and Gram negative). The clinical isolates were obtained from biomedical laboratory. *Escherichia coli, Vibrio cholerae* isolated in contaminate water, *Vibrio cholerae*, *Salmonella typhimirium* isolated in contaminate fish and *Salmonella typhimirium* isolated in contaminate salad were used. Serotype strains were: *Bacillus cereus* ATCC 9144, *Escherichia coli* ATCC 25922, *Escherichia coli* CPI 105182, *Proteus mirabilis* ATCC 35659, *Shigella dysenteriae* CPI 5451, *Staphylococcus aureus* ATCC 6538. Before testing, pure cultures were realized with all strains using Mueller Hinton (MH) Agar and tryptic soy Broth. Inoculate were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard.

Antibacterial Tests

Determination of anti-bacterial activity by agar diffusion method

The sensitivity of different bacterial strains to various fractions of extracts was measured by using disc diffusion method[8, 9]. Petri plates containing Mueller- Hinton/Nutrient agar were spread with 0.2 mL of the inoculum. 6 mm diameter of sterile Whatman filter paper were soaked with 10µL of extract (20 mg/mL) and deposited in plates. The plates inoculated with different bacteria were incubated at 37°C and 40°C for *E. coli* strains up to 24 h and diameter of any

resultant zone of inhibition was measured. For each combination of extract and the bacterial strain, the experiment was performed in triplicate and repeated twice. The bacteria with a clear zone of inhibition of more than 12 mm were considered to be sensitive. Sensitivity of different bacterial strains to DMSO was measured to evaluate this solvent toxicity. Anti-bacterial activities of different plant extracts was compared with three commonly employed antibiotics ampicillin (10 µg/disc), ciprofloxacin (10 µg/disc), and gentamicin (10 µg/disc).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of anti-bacterial (sensitive bacteria) activity was determinate with serial dilution technique, using 96-well microplates[10, 11]. In each colon of well 100µL of sterile MH broth and 100µL of sterile extract (20mg/mL) were put only in the first line. Successively dilutions permit to obtain extracts concentration between 20 and 0,325mg/mL. 10µL of each bacterial culture was added singly to each well. The density of bacteria was standardized using McFarland 0.5 turbidity standard. The plates were covered and incubated overnight (18 hours) at 37 °C and 44°C for *E. coli* strains. To indicate bacterial growth, 50 µL of 0.2 mg/mL p-iodonitrotetrazolium (INT) was added to each well and the plates incubated at 37 °C and 44°C for 30 min. Bacterial growth in the wells was indicated by a red color, whereas clear wells indicated inhibition by the tested substances. This assay was repeated three times.

Phytochemical investigation

Preliminary screening

Secondary metabolites such as polyphenols, tannins, flavonoids, coumarins, cardenolids, saponins, anthracenosids, sterols and triterpenes presence were determined by using methods as described by Ciulei[12] and Zaheer[13].

Total Phenolics Content

Total polyphenols were determined by using Folin-Ciocalteu method as described by Guenné[14] with some modifications. 96-well microtitre plate was used. 25 µL of extracts (0.1 mg/mL) were mixed with 105 µL of Folin- Ciocalteu reagent (0.2 N). 5 min later, 100 µL of Na_2CO_3 (75 g/L) were added in each well. After 2 h incubation in the dark at room temperature, the absorbances were measured at 760 nm against a blank (0.5 mL Folin-Ciocalteu reagent + 1 mL Na_2CO_3) using a UV/visible light spectrophotometer (Epoch 251465, Biotek Instruments, U.S.A.). The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (y= 0.0249x; R^2 = 0.99).The results were expressed as mg of gallic acid equivalents (GAE)/100 mg of extract.

Total Flavonoids Content

The total flavonoids content were estimated according to the Dowd method as adapted by Lamien-Meda[15] with some modifications. 75 µL of $AlCl_3$ (2%) were mixed with 75 µL of methanolic extract solution (0.1 mg/mL). After 10 min, the absorbances were measured at 415 nm against a blank (mixture of 75 µL methanolic extract solution and 75 µL methanol) on a spectrophotometer and compared to a quercetin calibration curve (y= 0.0289x 0.0036; R^2 = 0.99). The experiments were carried out in triplicate. The amounts of flavonoids in plant extracts were expressed as mg of quercetin equivalents (QE)/100 mg of extract.

Total Flavonols Content

The total flavonols content were determined as described by Abarca[16] method with some modifications. Aliquots were prepared by mixing of 75 µL ethanolic extract solutions (1 mg/mL) and 75 µL of aqueous $AlCl_3$ (20%). The absorbances were read at 425 nm after 10 min incubation against a blank (mixture of 75 µL ethanolic extract solutions and 75 µL of ethanol) on a spectrophotometer. All determinations were carried out in triplicate. A standard calibration curve (y = 0.0353x; R^2 = 0.99) was plotted using quercetin (0-50 µg/mL). The results were expressed as mg of quercetin equivalents (QE)/100 mg of extract.

Total Tannins Content

The total tannins contents were determined as described by European community[17]. In eppendorf tube, 20 µL of aqueous extract, 100µL of distilled water, 20 µL of ammonium ferric citrate (3.5 g/L) 24h older and 20 µL of ammoniac (8 g/L) were mixed. After 10 min, the absorbances of samples were measured at 525 nm against a blank (20 µL aqueous extract, 20 µL ammoniac + 100 µL distilled water) on a spectrophotometer and compared to tannic acid calibration curve (y= 0.0011x + 0.2236; R^2 = 0.99). The data obtained was the mean of three determinations. The results were expressed as mg of tannic acid equivalents (TAE) per 100 mg of extract (mg TAE/100 mg of extracts).

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviations (SD); Tukey's test was used to determine level of significance of all results obtained on XLSTAT 7.1. Results were regarded as significant at $p< 0.05$.

RESULTS AND DISCUSSION

Anti-bacterial activity

Inhibition zone diameters

The diameters of inhibition zones (Figure 1, Figure 2 and Figure 3) vary with the type of fraction and the bacterial strain tested. Best inhibition diameters of *V. cholerae* (fish) (14.67mm), *E. coli* (14.33) *and E. coli* (CPI 105182) were obtained with the crude extract of *V. colorata*. Similarly, hexane fraction of the same species gave the best result against *B. cereus* (ATCC: 9144), with a diameter of inhibition of 16.00 mm. The dichloromethane fraction of *E. alba* presented the best diameters of inhibition against *S. typhimurium* (salad) (14mm), *E. coli* (ATCC 25922) (13mm) and *S. aureus* (ATCC 6538) (15mm). The fractions of ethyl acetate and butanol of this same species gave the best inhibition zone diameters against *P. mirabilis* (ATCC 35659) (15.33mm), *V. cholerae* (water) (14.33). The best inhibition diameters of *B. cereus* (ATCC 9144) (16.33), *S. typhimurium* (fish) (15.33mm) were obtained with the crude extract of *C. americanum.* The ethyl acetate fraction of this plant also allowed the best results against *S. dysenteriae* (CPI 5451). Among the antibiotics tested, ampicillin presented the best activity: It was 2 to 3 times more active than the plant extracts. DMSO had no toxicity on the bacterial strains tested.

Minimum inhibitory concentration (MIC)

For strains susceptible to the extracts, values of minimum inhibitory concentrations (MIC) are shown in Table 1. For *V. cholerae* (fish), best values of MIC (2.5mg/mL) were obtained with the butanol fraction of *C. americanum*, dichloromethane and the butanol fractions of *V. colorata.* Against *E. coli* (CPI 105182) and *B. cereus* (ATCC 9144), the best MIC values (0.31mg/mL and 0.626mg/mL) were obtained respectively with ethyl acetate and butanol fractions of *C. americanum*. For *E. coli*, the best value of MIC (2.5mg/mL) was obtained with the crude extract and ethyl acetate fraction of *E. alba*. The butanol fraction of the same plant has presented the best values of MIC (1.25mg/mL; 1.12mg/mL; 1.25mg/mL; 0.62mg/mL) respectively against *S. typhimurium* (salad), *P. mirabilis* (ATCC 35659), *S. aureus* (ATCC: 6538) and *V. cholerae* (water). For *E. coli* (ATCC 25922) the best MIC (2.5mg/mL) was obtained with hexane fraction of *V. colorata*. For *S. typhimurium* (fish) the best MIC was obtained with the crude extract of *C. americanum*. Of all the extracts tested for antibacterial activity, the butanol fraction of *E. alba* presented the best activity.

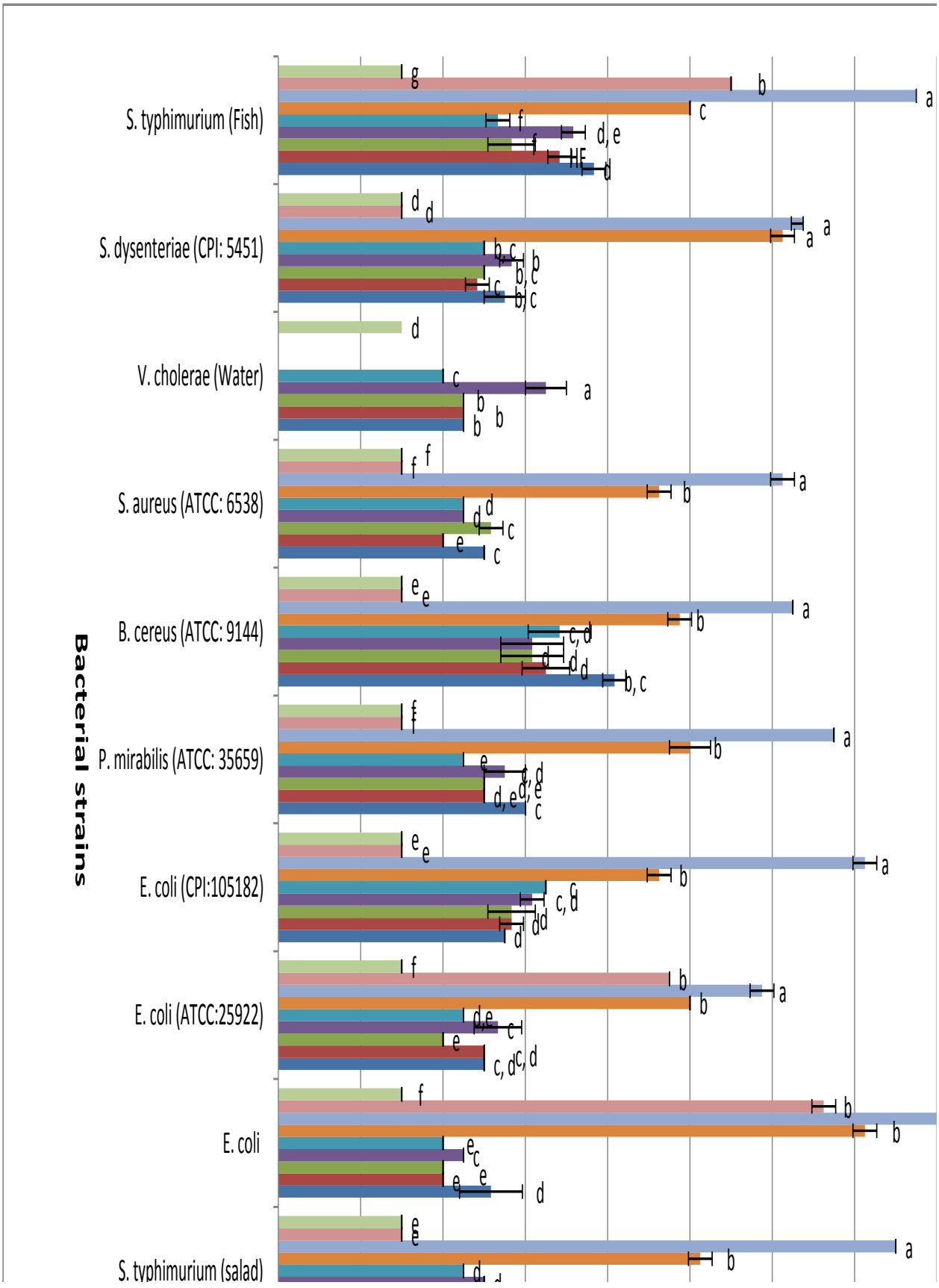

Figure 1: Strains inhibition zone diameters of *C. americanum* extracts

Result within each bar of strains with different letters (a - g) differs significantly ($p < 0.05$).

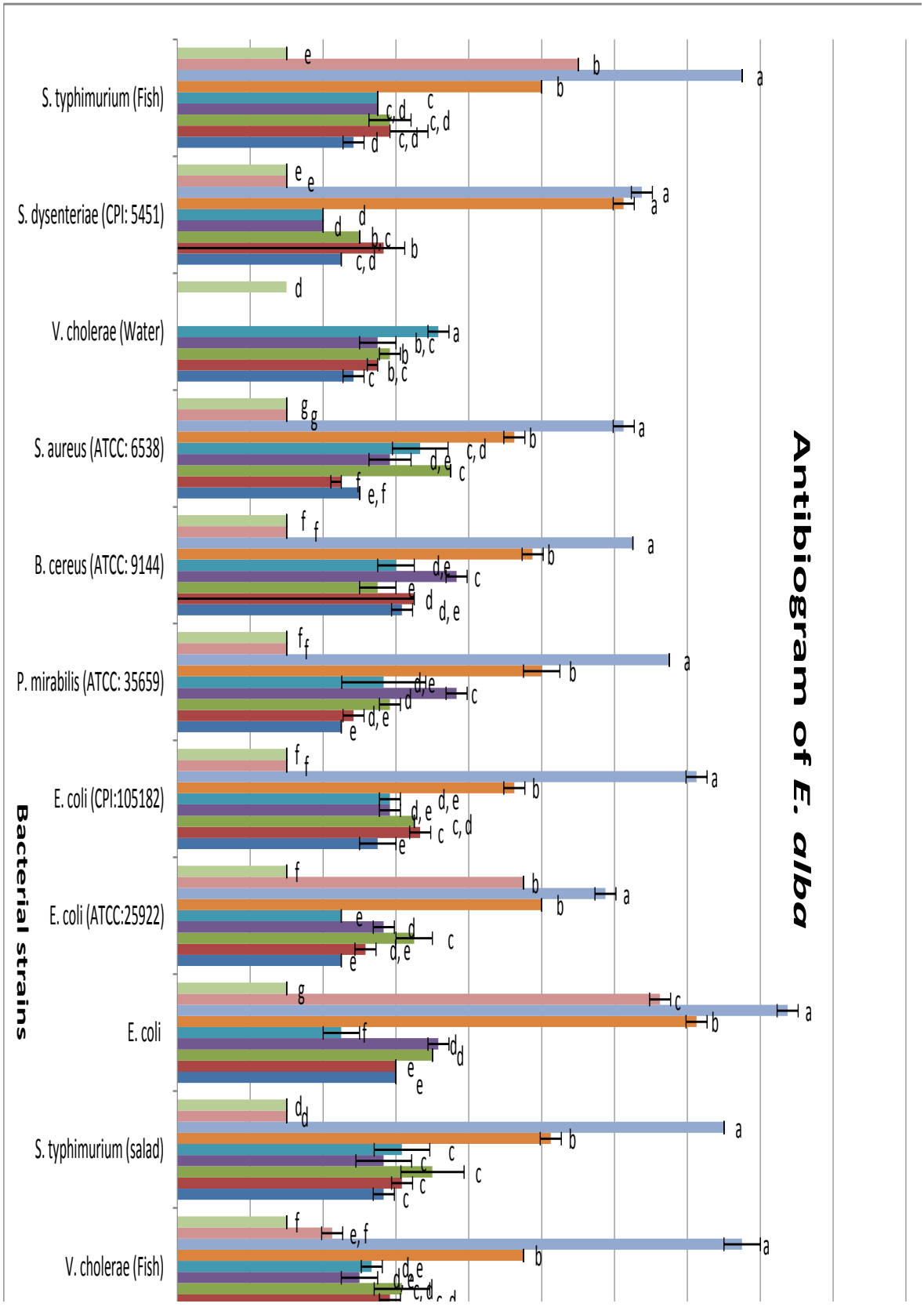

Figure 2: Strains inhibition zone diameters of *E. alba* extracts

Result within each bar of strains with different letters (a - g) differs significantly ($p < 0.05$).

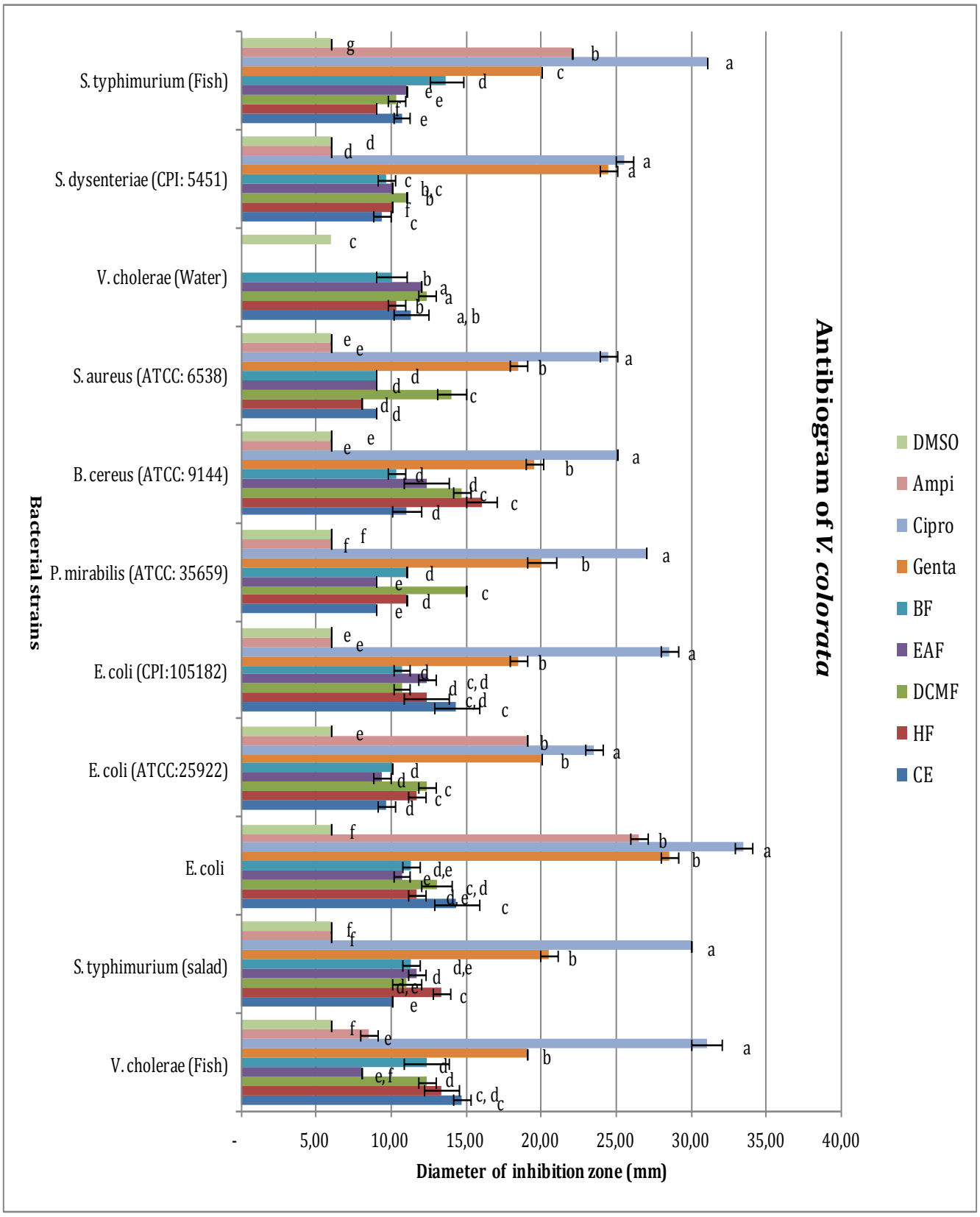

Figure 3: Strains inhibition zone diameters of *V. colorata* extracts

Result within each bar of strains with different letters (a - g) differs significantly ($p < 0.05$).

Table 1: Minimum inhibitory concentration (MIC)

Plants	Extracts	*V. cholerae* (fish)	*V. cholerae* (water)	*E. coli*	*E. coli (ATCC:25922)*	*E. coli (CPI:105182)*	*P. mirabilis (ATCC: 35659)*	*S. dysenteriae (CPI: 5451)*	*S. aureus (ATCC: 6538)*	*S. typhimurium* (salad)	*B. cereus (ATCC: 9144)*	*S. typhimurium* (fish)
		Gram -	*Gram -*	*Gram -*	*Gram -*	*Gram -*	*Gram -*	*Gram -*	*Gram +*	*Gram +*	*Gram +*	*Gram +*
C. americanum	CE	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	Nd	>20 mg/mL	**2,5 mg/mL**	>20 mg/mL	**1.25 mg/mL**
	HF	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL
	DCMF	>20mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/ml	>20 mg/mL	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL
	EAF	>20 mg/mL	**1.25 mg/mL**	>20 mg/mL	>20 mg/mL	**0.625 mg/mL**	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	**0.3125 mg/mL**	**2.5 mg/mL**
	BF	**2.5 mg/mL**	>20 mg/mL	>20 mg/mL	>20 mg/mL	0.625 mg/mL	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	**0.3125 mg/mL**	>20 mg/mL
E. alba	CE	**5 mg/mL**	>20 mg/mL	**2.5 mg/mL**	>20 mg/ml	>20 mg/mL	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	**2.5 mg/mL**	>20 mg/mL
	HF	>20 mg/mL	>20 mg/mL	**5 mg/mL**	>20 mg/mL	**5 mg/mL**	>20 mg/mL	Nd	>20 mg/mL	**5 mg/mL**	**5 mg/mL**	>20 mg/mL
	DCMF	**10 mg/mL**	>20 mg/mL	**5 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**5 mg/mL**	Nd	**5 mg/mL**	**5 mg/mL**	>20 mg/mL	>20 mg/mL
	EAF	>20mg/mL	>20 mg/mL	**2.5mg/mL**	>20 mg/mL	>20 mg/mL	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	**2.5 mg/mL**	>20 mg/mL
	BF	>20 mg/mL	**0.625 mg/mL**	>20 mg/mL	>20 mg/mL	>20 mg/mL	**1.25 mg/mL**	Nd	**1.25 mg/mL**	**1.25 mg/mL**	**0.3125 mg/mL**	**2.5 mg/mL**
V. colorata	CE	**5 mg/mL**	>20 mg/mL	**5mg/mL**	>20 mg/mL	**2.5 mg/mL**	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	>20 mg/ml	>20 mg/mL
	HF	**20 mg/mL**	>20 mg/mL	>20 mg/mL	**2.5 mg/mL**	**20 mg/mL**	>20 mg/mL	Nd	>20 mg/mL	**20 mg/mL**	**20 mg/mL**	>20 mg/mL
	DCMF	**2.5 mg/mL**	**2.5 mg/mL**	**5 mg/mL**	>20 mg/mL	>20 mg/mL	**5 mg/mL**	Nd	**10 mg/mL**	>20 mg/mL	**2.5 mg/mL**	>20 mg/mL
	EAF	>20 mg/mL	**10 mg/mL**	>20 mg/mL	>20 mg/mL	**5 mg/mL**	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	**5 mg/mL**	>20 mg/mL
	BF	**2.5 mg/mL**	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	>20 mg/mL	Nd	>20 mg/mL	>20 mg/mL	>20 mg/mL	**5 mg/mL**

CE: Crude Extract; HF: Hexan Fraction; DCMF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanol Fraction ; Nd: Not determinate

Phytochemical studies

Preliminary screening

A preliminary phytochemical screening allowed detecting the secondary metabolites such as polyphenols, tannins, flavonoids, coumarins, cardenolids, saponins, anthracenosids, sterols and triterpenes (table 2). For these preliminary test carried out, *E. alba* and *V. colorata* had similar phytochemical profiles and they differs from C*. americanum* by anthracenosids presence. The presence such these well-known bioactive compounds justify the medicinal effects of these traditional medicine species of Burkina Faso[4, 18].

Polyphenols contents

The total phenolics, total flavonoids, total flavonols and total tannins contents of crude extracts and fractions of the three species of *Asteraceae* are shown in Table 3.

Table 2: preliminary phytochemical screening

Tested material		Extraction yield (%)	Positive tests for	Negative tests for
C americanum	CE	8.00	Flavone , saponins, tannins and/or polyphenols, Emodols (anthracenosids), cardenolids, sterols/ triterpene	Coumarins
	HF	0.76	Cardenolids, sterols/ triterpene	Flavone, saponins, tannins and/or polyphenols, Emodols (anthracenosids), coumarins
	DCMF	0.99	Cardenolids, sterols/ triterpene	Flavone, saponins, tannins and/or polyphenols, Emodols (anthracenosids), coumarins
	EAF	1.06	Saponins, tannins and/or polyphenols, Emodols (anthracenosids), cardenolids, sterols/ triterpene	Flavone, coumarins
	BF	4.77	Saponin, tannins and/or polyphenols, Emodols (anthracenosids), cardenolids, sterols/ triterpene	Flavone, coumarins
E. alba	CE	6.41	Flavone, saponins, tannins and/or polyphenols, cardenolids coumarins, sterols/ triterpene	Emodols (Anthracenosids)
	HF	1.66	Cardenolids, sterols/ triterpene	Flavone, saponins, tannins and/or polyphenols, Emodols (anthracenosids), coumarins
	DCMF	0.52	Saponins, cardenolids	Flavone, tannins and/or polyphenols, Emodols (anthracenosids), coumarins, sterols/ triterpene
	EAF	1.67	Flavone, saponin, tannins and/or polyphenols, cardenolids, coumarins, sterols/ triterpene	Emodols (anthracenosids)
	BF	2.50	Saponins, tannins and/or polyphenols, cardenolids, coumarins	Flavone, anthracenosids, sterols/ triterpene
V. colorata	CE	15.66	Flavone, saponins, tannins and/or polyphenols, cardenolids, coumarins, sterols/ triterpene	Emodols (anthracenosids)
	HF	0.40	Cardenolids, sterols/ triterpene	Flavone, saponins, tannins and/or polyphenols, Emodols (anthracenosids), Coumarins
	DCMF	7.18	Saponins, cardenolids	Flavone, tannins and/or polyphenols, Emodols (anthracenosids), coumarins, sterols/ triterpene
	EAF	2.49	Flavone, saponins, tannins and/or polyphenols, cardenolids, coumarins, sterols/ triterpene	Emodols (anthracenosids)
	BF	5.75	Saponins, tannins and/or polyphenols, cardenolids, coumarins, sterols/ triterpene	Flavone, Emodols (anthracenosids)

Table 3: polyphenols contents of extracts

Plants		Fractions	Total phenolic content (mg GAE/100 mg of extract)	Total flavonoids content (mg QE/100 mg of extract)	Total flavonols content (mg QE/100 mg of extract)	Total tannins content(mg TAE/100 mg extracts)
C. americanum		CE	79.09 ± 0.80^{def}	13.54 ± 0.44^{f}	1.50 ± 0.08^{e}	21.31 ± 1.75^{d}
		HF	74.89 ± 0.76^{g}	9.86 ± 0.38^{g}	0.23 ± 0.01^{i}	19.02 ± 1.00^{de}
		DCMF	77.52 ± 0.16^{f}	41.98 ± 0.66^{b}	4.92 ± 0.14^{a}	1.95 ± 0.09^{h}
		AEF	85.50 ± 1.10^{bc}	24.71 ± 0.81^{e}	3.93 ± 0.37^{b}	9.07 ± 0.81^{h}
		BF	85.65 ± 1.77^{bc}	24.03 ± 0.88^{e}	3.68 ± 0.07^{hi}	23.01 ± 1.05^{g}
E. alba		CE	80.98 ± 1.15^{d}	15.09 ± 1.00^{f}	0.98 ± 0.13^{f}	60.67 ± 1.15^{a}
		HF	77.94 ± 0.56^{ef}	23.47 ± 0.93^{e}	0.88 ± 0.04^{fg}	24.40 ± 0.09^{c}
		DCMF	84.11 ± 0.60^{c}	33.54 ± 0.34^{c}	3.26 ± 0.15^{c}	13.98 ± 0.88^{f}
		AEF	84.24 ± 0.62^{c}	24.89 ± 0.38^{e}	1.83 ± 0.10^{e}	10.31 ± 0.69^{g}
		BF	88.88 ± 0.70^{a}	31.33 ± 0.44^{d}	2.67 ± 0.12^{d}	16.70 ± 0.14^{ef}
V. colorata		CE	80.20 ± 0.85^{de}	15.11 ± 0.61^{f}	0.51 ± 0.00^{ghi}	49.33 ± 2.31^{b}
		HF	78.09 ± 0.66^{ef}	49.48 ± 0.92^{a}	2.50 ± 0.02^{d}	3.67 ± 0.09^{h}
		DCMF	85.37 ± 0.63^{bc}	10.47 ± 0.82^{g}	0.67 ± 0.07^{fgh}	20.02 ± 0.99^{d}
		AEF	86.69 ± 0.94^{abc}	6.55 ± 0.20^{h}	0.51 ± 0.03^{ghi}	10.82 ± 0.50^{g}
		BF	86.96 ± 0.44^{ab}	10.09 ± 0.67^{g}	0.95 ± 0.03^{f}	0.88 ± 0.14^{h}

The butanol fraction and crude extract of *E. alba* contains the highest content in total phenolics (88.88 ± 0.70 mg GAE/100 mg of extract) and total tannins (60.67 ± 1.15 mg TAE/100 mg extracts). For total flavonols, the best content (4.92 ± 0.14 mg QE/100 mg of extract) was obtained with *C. americanum* dichloromethane fraction. *V. colorata* hexane fraction (with 49.48 ± 0.92 mg QE/100 mg of extract) presented the best content in total flavonoids. *E. alba* butanol fraction presented the best anti-bacterial activity; that could be explained by its higher content of phenolic compounds (flavonoids and phenol acids)[7, 19]. This activity could be also justified by the presence of 4 triterpenoids (eclalbasaponin I, eclalbasaponin II, eclalbasaponin III and eclalbasaponin V) isolated in this fraction by Lee[20]. Isolated compounds (vernolide and its derivates) from *V. colorata* extract have been shown (Rabe[18]) to possess anti-bacterial activities on some bacteria strains like *S. aureus* (ATCC 12600), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 11775), and *Klebsiella pneumonia* (ATCC 13883). These compounds in addition to the phenolic compounds could also justify growth inhibitor of susceptible strains here tested. However vernolide and its derivatives have high toxicity[21], so there is need to investigate new anti-bacterial compounds this plant. Antioxidant properties of phenolic compounds are an advantage for further anti-bacterial compounds investigation. Thus the three plants presented interesting phenolic contents. In Burkina Faso *C americanum* is used in the treatment of metabolic diseases only; these results shows that it could as be used in bacterial diseases especially as this plant has practically no toxicity.

CONCLUSION

In this study; anti-bacterial activities and phenolic compounds content of raw and fractioned extracts of three plants were evaluated. Compared to pure references antibiotics used, extracts of

plants presented good anti-bacterial activities. These activities are at least partly due to the phenolic compounds. The plants also presented good antioxidant phenolic compounds contents; that justify the uses of the plants for anti-bacterial purposes. *C. americanum* extracts was known for its antioxidant properties but this study reveals the presence of anti-bacterial compound in its extracts. *E. alba* species which presented the best anti-bacterial activity and interesting phenolic compound contents is a potential source of bioactive compounds. It will be also interesting to elucidate structures of the anti-bacterial molecules.

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REFERENCES

1. Schwaiger K, Helmke K, Hölzel CS, Bauer J. Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs. supermarket). International Journal of Food Microbiology 2011; 148: 191- 196.
2. World Health Day 2011. Combat Antimicrobial Resistance. WHO 2011; http://www.who.int/world-health-day/2011.
3. Jäger A.K., Hutchings A., van Staden J. Screening of Zulu medicinal plants for protaglandin-synthesis inhibitors. J. of Ethnopharm. 1996; 52: 95-100.
4. Nacoulma O. G. 1996. Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: Cas du plateau central, Tome II. Thèse de doctorat d'état ès sciences naturelles, Université de Ouagadougou; Ouagadougou.
5. Lengani A., Lompo L.F., Guissou I.P. and Nikiema Jb. Médecine traditionnelle et maladies des reins au Burkina faso. Néphrologie & Thérapeutique 2010; 6 (1): 35-39.
6. Nadembega P., Boussim J.I., Nikiema Jb., Poli F. and Antognonia F. Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. J. Ethnopharmacol. 2011; 133 (2): 378–395.
7. Konaté K., Kiendrébéogo M., Ouattara M.B., Souza A., Lamien-Meda A., Nongasida Y., Barro N., Millogo-Rasolodimby J. and Nacoulma O.G. Antibacterial Potential of Aqueous Acetone Extracts From Five Medicinal Plants used Traditionally to Treat Infectious Diseases in Burkina Faso. Curr. Res. J. Biol. Sci. 2011; 3(5): 435-442.
8. Bauer A.W., Kirby W.M.M., Sherris J.C., Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 43: 493-496.
9. Paulsamy S. and Thambiraj J. Evaluation of antimicrobial efficacy of the folklore medicinal plant, *Acacia caesia* (L.) Wild. Asian J Pharm Clin Res 2011; 4 (3): 103-105.
10. Eloff J.N. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 1998; 64: 711-713.
11. NCCLS-National Committee for Clinical Laboratory Standards 2001. Performance standard for anti-microbial susceptibility testing: eleventh informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standard, Wayne, PA, USA.
12. Ciulei I. Methodology for analysis of vegetable drugs. Bucharest Ministry of Chemical Industry; 1982.
13. Zaheer Z., Konale A.G., Patel K.A., Khan S., Ahmed R.Z. Comparative phytochemical screening of flowers of *Plumeria alba* and *Plumeria rubra*. Asian J Pharm Clin Res 2010; 3 (4): 88- 89.
14. Guenné S., Ouattara N., Hilou A., Millogo J. F. and Nacoulma O. G. Antioxidant, enzyme inhibition activities and polyphenol contents of three asteraceae species used in burkina faso traditionally medicine. Int J Pharm Pharm Sci 2011; 3 (5): 524-528.
15. Lamien-Meda A., Lamien, C.E., Compaoré, M.M.Y., Meda, R.T.N., Kiendrebeogo, M., Zeba, B., Millogo, J.F. and Nacoulma O.G. 2008. Polyphenol content and antioxidant activity of fourteen wild elible fruits from Burkina Faso. Molecules, 13:581-594.
16. Abarca N.A., Campos M.G., Reyes J.A.A., Jimenez N.N., Corral J.H., Valdez S.G. Antioxidant activity of phénolic extract of monofloral honeybee-collected pollen from mesquite (*Prosopis julifloral*, Leguminoseae). Journal of Food Composition and Analysis 2007; 20 (2): 119-124.
17. European Community. Méthode de référence pour le dosage des tannins. *Journal officiel des Communautés Européennes* 2000; 197: 18-20.
18. Rabe T., Mullholland D., Staden J. Isolation and identification of antibacterial compounds from *Vernonia colorata* leaves. J. of Ethnopharm 2002; 80 (1): 91-94.
19. Kilani-Jaziri S., Bhouri W., Skandrani I., Limem I., Chekir-Ghedira L., Ghedira K. Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of *Cyperus rotundus* extracts. South African Journal of Botany 2011; 77: 767- 776.
20. Lee M.K., Ha N.R., Yang H., Sung S.H., Kim G.H., Kim Y.C. Antiproliferative activity of triterpenoids from Eclipta prostrata on hepatic stellate cells. Phytomedicine 2008; 15: 775-780.
21. Chukwujekwu J.C., Lategan C.A., Smith P.J., Van Heerden F.R., Van Staden J. Antiplasmodial and cytotoxic activity of isolated sesquiterpene lactones from the acetone leaf extract of *Vernonia colorata*. South African Journal of Botany 2009; 75: 176- 179.