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Antibacterial and Phytochemical studies of three *Acanthaceae* species used in Burkina Faso traditional medicine

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ABSTRACT

New and innovative antibacterial drugs research from plants is a best strategy to overcome the world problem about bacterial diseases. The aim of this study was to evaluate the *in-vitro* antibacterial activity and the phytochemical profile of various extracts from *Hygrophila auriculata* (Schumach.) Heine, *Nelsonia canescens* (Lam) Spreng and *Peristrophe bicalyculata* (Retz.) Nees; three acanthaceae species widely used in folk medicine of Burkina Faso. The inhibition diameter determination of the eleven bacteria strains and the Minimum Inhibition Concentration (MIC) of the plants extracts and fractions were evaluated using standards methods. All extracts were also evaluated for their phenolic contents. The results showed that all extracts and fractions exhibited good bacteria inhibition and have a wealth of phenolic compounds. Comparing the MIC of extracts and fractions, *E. coli* (CPI: 105182) strain was best inhibited and *N. canescens* butanol fraction presented the best activity. As regard phytochemical profile, highest values of total phenolics, total flavonoids, total flavonols and total tannins were obtained with *N. canescens* fractions. The results could justify the traditional used of these species against infectious diseases. This study shows that these three medicinal plants and especially *N. canescens* are good candidate for isolate new et innovative antibacterial compounds.

INTRODUCTION

Bacterial diseases are a major health problem because they are responsible of numerous deaths per day worldwide. The world health organization (2002) and UNAIDS (2007) reported that between 14 and 17 million people die each year to the infectious diseases. Infectious diseases cause enormous mortality in developing countries because of the extreme poverty of the people compared to developed countries. This situation is aggravated by the lack of suitable vaccine, inaccessibility and/or lack of antibiotics and the emergence of antibiotic-resistant strains. Historically, the researchers made many efforts to discover new antibacterial compounds. One of important discovery source of these antibacterial drugs is medicinal plants throughout folk medicines. In addition it is known that contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al.*, 1999). In Burkina Faso, *Acanthaceae* is one of the greatest families most used in traditional medicine; *Hygrophila auriculata* (Schumach.) Heine, *Nelsonia canescens* (Lam) Spreng and *Peristrophe bicalyculata* (Retz.) Nees are three medicinal plants of this family very used traditionally to treat various infectious diseases such as diarrheal diseases, cholera, typhoid fever, tuberculosis. Previous antibacterial activities showed that leaves extract of *Hygrophila auriculata* for the period from September to October exhibited a good antibacterial activity on *Escherchia coli* (NCIM N^{O} 2341), *Staphylococcus aureus* (NCIM N^{O} 2654), *Bacilus subtilis* (NCIM N^{O} 2195) and *Pseudomonas aeruginosa* (NCIM N^{O} 2914) (Patra *et al.*, 2009). Leaves petroleum ether, chloroform, alcohol and aqueous extracts showed good antibacterial effects (Patra *et al.*, 2008). Mohapatra *et al.*, 2011 Studies showed that chloroform extract is very effective against *Vibrio cholerae* 811, *Shigella boydii* 81 and *Bacillus licheniformis* 10341 compared to other bacteria strains *Escherchia coli* L. row, *Shigella sonnei* 2, *Salmonella typhi* 59, *Staphylococcus aureus* 29737, *Vibrio alginolyteus* , *Salmonella typhimurium* NCTC74, *Vibrio cholera* 854 and *Staphylococcus aureus* 29737.

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A study by Jani *et al.,* 2012 showed that stem methanol extract of *H. auriculata* is more active (MIC = 0.5 mg/mL) on *B. subtilis* bacterial strain, while leaves and flowers methanol extracts showed good average inhibitory concentration (0.5 mg/mL) on *Proteus vulgaris*. The ethanolic extract of *Peristrophe bicalyculata* was more effective against *Escherchia coli* (with a largest inhibition zone = 18 ± 0.8 mm), *Bacillus cereus* and *Salmonella typhi* (Janakiraman *et al.*, 2012). The aqueous extract (20 mg / mL) of the plant is effective against *Escherchia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Khan *et al.*, 2011). A yellow-brown essential oil can be extracted by distillation, shows in vitro activity against the growth of various strains of Mycobacterium tuberculosis (Burkill, 1985). The leaves ethanolic macerated of *Peristrophe bicalyculata* showed greater inhibition *in vitro* against *Staphylococcus aureus*, *Klebsiella spp, Pseudomonas aeruginosa*, *Aspergillus Niger, Rhizopus stolonifer* and *Aspergillus clavatus* (Giwa *et al.,* 2010). In the best of our knowledge, there is no scientific information concerning the *in vitro* antibacterial activities in relation with polyphenolic contents of these three medicinal plants. This study aimed to evaluate the *in vitro* antibacterial activities and polyphenol contents of plants extracts.

MATERIALS AND METHODS

Chemicals

To carry out our different activities (phytochemical screening and antibacterial activities), we used solvents and various classic reagents. All reagents were of analytical grade. Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium hydroxide, gallic acid, quercetin, aluminium trichloride ($AlCl_3$), hydrochloric acid, magnesium chloride, were purchased from Sigma Aldrich chemie (Steinheim, Germany); ammonium ferric citrate and potassium persulfate were supplied by Fluka chemie (Buchs, Switzerland); sulfuric acid, acetic anhydride, ferric trichloride, chloroform, ethanol, methanol, was sourced from Probalo (Paris, France); p-iodonitrotetrazolium chloride (INT) were sourced from Sigma–Aldrich (Germany); ascorbic acid and tannic acid were supplied by labosi (Paris, France).

Plants materials

Hygrophila auriculata, *Nelsonia canescens* and *Peristrophe bicalyculata* were collected in August 2008 in Loumbila, 15 Km north of Ouagadougou, capital of Burkina Faso. The plants were identified by Prof. Millogo- Rasolodimby botanist at the plant Biology Department of the University of Ouagadougou. Voucher specimens with accession numbers ID 10259, ID 10152 and ID 10320 respectively for *Hygrophila auriculata*, *Nelsonia canescens* and *Peristrophe bicalyculata* were deposited at the Herbarium of the Laboratory of plants .

Preparation of Extracts

Whole plants of *Hygrophila auriculata*, *Nelsonia canescens* and *Peristrophe bicalyculata* were dried at room temperature and ground to fine powder. Twenty five grams (25g) 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 crude extracts obtained were fractionated by solvents of increasing polarity respectively 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. They were: *Escherichia coli, Vibrio cholera* isolate in contaminate water, *Vibrio cholera, Salmonella typhimirium* isolate in contaminate fish and *Salmonella typhimirium* isolates in contaminate salad. Sereotyped strains were: *Bacillus cerus* ATCC 9144, *Escherichia coli* ATCC 25922, *Escherichia coli* CPI 105182, *Proteus mirabilis* ATCC 35659, *Shigella dysenteria* CPI 5451, *Staphylococcus aureus* ATCC 6538. Before testing, pure cultures were realized with all strains using Mueller Hinton Agar and tryptic soy Broth. Inoculate were prepared by adjusting the turbidity of the suspension to match the 0.5 Mc Ferland standard.

Antibacterial tests

Determination of antibacterial activity by agar diffusion method

Sensitivity of different bacterial strains to various fractions of extracts was measured in using disc diffusion (Bauer *et al.,* 1966; Chew *et al.,* 2011). The plates containing Mueller-Hinton/Nutrient agar were spread with 0.2 ml of the inoculum. 6mn diameter of sterile Whatman filter paper were soaked with 10µl of extract (20mg/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. Antibacterial activity of different plant extracts was compared with three commonly employed antibiotics *viz*. ampicillin (10 µg/disc), ciprofloxacin (10 µg/disc), and gentamicin (10 µg/disc).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of antibacterial (sensitive bacteria) activity was determinate with serial dilution technique, using 96-well microplates (Eloff, 1998; NCCLS, 2001). 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 violet (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, saponins, anthracenosids, sterols and triterpenes presence were determined by using methods as described by Ciulei (1982).

Total Phenolics Content

Total polyphenols were determined by using Folin-Ciocalteu method as described by Singleton *et al.,* (1999) 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 *et al.,* (2008) 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 *et al.,* (2007) 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 (2000). In eppendoff 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 (8g/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

Antibacterial activity

Inhibition zone diameters

The result of the antibacterial efficacy of Crude extract; Hexane, Dichloromethane, Ethyl Acetate and Butanol Fractions of *H. auriculata, N. canescens* and *P. bicalyculata* is showed in Tables 1, 2 and 3. Among the tested extracts and fractions, the best inhibition zone diameter of *V. cholerae* (Fish) (14mm) was obtained with *H. auriculata* Hexane Fraction; the best *S. typhimurium* (salad) (25mm) *N. canescens* Hexane fraction; the bests inhibition zone diameters of *E. coli* boromo (12mm) were obtained with *H. auriculata, N. canescens* and *P. bicalyculata* Hexane fractions; the bests inhibition zone diameters of *E. coli* (ATCC: 25922) (14.67mm) were obtained with *H. auriculata* Dichloromethane fraction and *P. bicalyculata* Butanol fraction; the best inhibition zone diameter of *E. coli* (CPI: 105182) (15.33mm) was obtained with *P. bicalyculata* Dichloromethane fraction; the best inhibition zone diameter of *P. mirabilis* (ATCC: 35659) (28.33mm) was obtained with Crude extract of *P. bicalyculata;* the best inhibition zone diameter of *B. cerus* (ATCC: 9144) (11.67mm) was obtained with *H. auriculata* Butanol fraction, for *S. aureus* (ATCC: 6538) the best inhibition zone diameter (14.67mm) was obtained with *P. bicalyculata* Dichloromethane fraction; for *V. cholerae* (Water) the best inhibition zone diameter (14mm) was obtained with *N. canescens* Ethyl Acetate fraction; the best inhibition zone diameter of *S. dysenteria* (CPI: 5451) (11.67mm) was obtained with Crude extract of *H. auriculata* and finally for *S. typhimurium* (Fish) the best inhibition zone diameter

(12 mm) was obtained with *N. canescens* Ethyl Acetate fraction. Among the standards compounds (antibiotics) used in this test, Ampicillin presented the best activity on all strains compared to plant extracts and fraction while DMSO didn't have good antibacterial activity on bacterial strains.

Minimum inhibitory concentration (MIC)

The table 4 indicated the minimum inhibitory concentrations (MIC) of sensitive strains by plants extracts and fractions.

For the strain *V. cholerae* (Fish) bests MIC of 2.5 mg/mL were obtained with *N. canescens* Dichloromethane, Ethyl Acetate and Butanol Fractions. For *S. typhimurium (salad)* bests MIC were ranged in the following order *N. canescens* Butanol Fraction > *P. bicalyculata* Hexane and Dichloromethane Fractions > *H. auriculata* Dichloromethane Fraction and *P.bicalyculata* Ethyl Acetate fraction with respectively 0.625mg/mL, 1.25 mg/mL and 2.5 mg/mL. For *E. coli* (ATCC: 25922) the best MIC of 1.25 mg/mL was obtained with *H. auriculata* Dichloromethane Fraction; for *E. coli* (CPI: 105182) strain bests MIC of 0.3125 mg/mL were obtained with *H. auriculata*, *N. canescens* and *P. bicalyculata* Dichloromethane Fractions and with *N. canescens* Butanol fraction. In addition for the same strain, *P. bicalyculata* Ethyl Acetate and Butanol Fractions exhibited good MIC of 2.5 mg/mL; for *P. mirabilis* (ATCC: 35659) the MIC were ranged in the following order *N. canescens* Butanol fraction > *P. bicalyculata* Ethyl Acetate fraction > *H. auriculata* crude extract with respectively 0.625 mg/mL, 1.25 mg/mL and 2.5 mg/mL; and for *V. cholerae* (Water) MIC value of 2.5 mg/mL was obtained with Ethyl Acetate fraction *N. canescens*. We noted that *E. coli* (CPI: 105182) strain was best inhibited compared with the others bacterial strains and as regards all extracts and fractions antibacterial activities, *N. canescens* butanol fraction presented the best activity.

Table. 1: Strains inhibition zone diameters of crude and hexane fractions.

Bacterial Strains	Crude extract			Hexane fraction		
	H. auriculata	***N. canescens***	***P. bicalyculata***	***H. auriculata***	***N. canescens***	***P. bicalyculata***
A	$11\pm0^{c,d,e,f}$	$10\pm0^{e,f}$	$11.67\pm0.58^{c,d,e,f}$	14 ± 1^{b}	$12.67\pm0.58^{c,d,e}$	$12.67\pm1.15^{c,d,e}$
B	$8\pm0^{e,f}$	$8\pm0^{e,f}$	$8.33\pm0.58^{e,f}$	$11\pm1^{e,f}$	$25\pm2.89^{a,b}$	$17.67\pm1.53^{c,d}$
C	$10.67\pm0.58^{c,d}$	$9\pm0^{d,e}$	12.67 ± 0.58^{c}	$9.00\pm0^{d,e}$	9.33 ± 0.58^{d}	$10.67\pm1.15^{c,d}$
D	$9\pm1^{f,g}$	$13.67\pm0.58^{d,e}$	$9.33\pm0.58^{f,g}$	$11.00\pm1^{e,f}$	$11.33\pm1.15^{d,e,f}$	10.00 ± 0^{f}
E	$7\pm0^{g,h}$	$11.33\pm0.58^{d,e,f}$	$9\pm0^{f,g}$	$13.67\pm0.58^{c,d}$	$12.67\pm2.08^{c,d,e}$	$13.00\pm0^{c,d,e}$
F	$12.67\pm2.08^{c,d}$	$9\pm0^{e,f}$	28.33 ± 2.89^{a}	13.33 ± 0.58^{c}	$9.00\pm0^{e,f}$	$11.00\pm0^{c,d,e,f}$
G	$10\pm0^{c,d}$	$10.67\pm0.58^{c,d}$	$9.67\pm0.58^{c,d}$	$10.33\pm0.58^{c,d}$	9.33 ± 0.58^{d}	$11.33\pm0.58^{c,d}$
H	$12\pm1^{c,d,e,f}$	$9.67\pm0.58^{f,g,h,i}$	$10\pm1^{f,g,h}$	$7.00\pm0^{i,j}$	$7.00\pm0^{i,j}$	$13.67\pm0.58^{c,d}$
I	$11.67\pm0.58^{a,b}$	10.33 ± 1.53^{b}	10.67 ± 1.15^{b}	11.33 ± 1.15^{b}	9.67 ± 0.58^{b}	11.33 ± 0.58^{b}
J	11.67 ± 0.58^{b}	$8\pm0^{f,g}$	$8\pm0^{f,g}$	$9.00\pm0^{d,e,f}$	$10.33\pm0.58^{b,c,d}$	$8.67\pm0.58^{e,f}$
K	$10.67\pm1.15^{c,d}$	11.67 ± 0^{c}	11 ± 0^{c}	$8.00\pm0^{d,e}$	11.33 ± 0.58^{c}	11.67 ± 0.58^{c}

Table. 2: Strains inhibition zone diameters of Dichloromethane and ethyl Acetate fractions.

Bacterial Strains	DCM Fraction			Ethyl Acetate fraction		
	H. auriculata	***N. canescens***	***P. bicalyculata***	***H. auriculata***	***N. canescens***	***P. bicalyculata***
A	$11.33\pm0.58^{c,d,e,f}$	$12.67\pm2.89^{c,d,e}$	$10.00\pm1^{e,f}$	$12.00\pm0^{c,d,e}$	$12.67\pm0.58^{c,d,e}$	$11.33\pm1.15^{c,d,e,f}$
B	$13.00\pm1^{d,e}$	$10.67\pm0.58^{d,e,f}$	$15.67\pm2.52^{c,d}$	$8.00\pm0^{e,f}$	$11.00\pm1.73^{d,e}$	$13.00\pm0^{d,e}$
C	$9.67\pm0.58^{c,d}$	$10.33\pm0.58^{c,d}$	9.33 ± 0.58^{d}	$12.00\pm0^{c,d}$	$12.00\pm2.65^{c,d}$	$12.00\pm0^{c,d}$
D	$14.67\pm2.89^{c,d}$	$11.33\pm2.31^{d,e,f}$	$11.00\pm0^{e,f}$	$17.33\pm1.15^{b,c}$	$11.00\pm1^{e,f}$	$11.33\pm0.58^{d,e,f}$
E	$12.00\pm0^{d,e}$	$13.00\pm1^{c,d,e}$	15.33 ± 0.58^{c}	$11.67\pm0.58^{d,e,f}$	$10.67\pm2.31^{e,f}$	$12.00\pm1^{d,e}$
F	$11.33\pm0.58^{c,d,e,f}$	$10.33\pm0.58^{d,e,f}$	$10.00\pm0^{d,e,f}$	$8.67\pm0.58^{f,g}$	$11.67\pm0.58^{c,d,e}$	$12.00\pm0^{c,d}$
G	$10.33\pm0.58^{c,d}$	$11.00\pm0.58^{c,d}$	$9.67\pm0.58^{c,d}$	$11.00\pm0^{c,d}$	$10.33\pm1.53^{c,d}$	$10.67\pm1.15^{c,d}$
H	$7.00\pm0^{i,j}$	$13.33\pm3.21^{c,d,e}$	14.67 ± 0.58^{c}	$10.67\pm0.58^{e,f,g,h}$	17.67 ± 1.15^{b}	$11.00\pm0^{d,e,f,g}$
I	10.67 ± 0.58^{b}	10.00 ± 0^{b}	$11.67\pm0.58^{a,b}$	11.00 ± 1^{b}	14.00 ± 1^{a}	11.00 ± 1^{b}
J	$11.33\pm0.58^{b,c}$	$10.00\pm0^{c,d,e}$	$10.67\pm1.15^{b,c}$	$10.00\pm0^{c,d,e}$	$10.00\pm0^{c,d,e}$	$10.33\pm0.58^{b,c,d}$
K	11.67 ± 1.15^{c}	11.67 ± 0.58^{c}	$10.67\pm1.15^{c,d}$	$10.00\pm1^{c,d}$	12.00 ± 1.73^{c}	$10.00\pm0^{c,d}$

Table. 3: Strains inhibition zone diameters of Butanol fraction, Standards and Solvent.

Bacterial Strains	Butanol fraction			Standards			Solvent
	H. auriculata	***N. canescens***	***P. bicalyculata***	**Gentamicin**	**Ciprofloxacin**	**Ampicillin**	**DMSO**
A	$10,67\pm1,15^{d,e,f}$	$13,67\pm1,53^{c,d}$	$11,00\pm0^{c,d,e,f}$	19 ± 0^{b}	31 ± 1^{a}	$8,67\pm0,58^{f,g}$	6 ± 0^{g}
B	$10,00\pm1^{e,f}$	$12,00\pm0^{d,e}$	$9,00\pm0^{e,f}$	$20,33\pm0,58^{b,c}$	30 ± 0^{a}	6 ± 0^{f}	6 ± 0^{f}
C	$10,67\pm1,15^{c,d}$	$11,00\pm0^{c,d}$	$11,67\pm2,52^{c,d}$	$28,67\pm0,58^{b}$	$33,33\pm0,58^{a}$	$26,67\pm0,58^{b}$	6 ± 0^{e}
D	$10,67\pm0,58^{e,f}$	$9,67\pm0,58^{f}$	$14,67\pm1,15^{c,d}$	$20\pm0^{a,b}$	$23,33\pm0,58^{a}$	19 ± 0^{b}	6 ± 0^{g}
E	$11,33\pm0,58^{d,e,f}$	$12,00\pm0^{d,e}$	$13,00\pm1^{c,d,e}$	$18,67\pm0,58^{b}$	$28,33\pm0,58^{a}$	6 ± 0^{h}	6 ± 0^{h}
F	$11,67\pm0,58^{c,d,e}$	$12,00\pm0^{c,d}$	$11,00\pm0^{c,d,e,f}$	20 ± 1^{b}	27 ± 0^{a}	6 ± 0^{g}	6 ± 0^{g}
G	$11,67\pm0,58^{c}$	$7,00\pm0^{e}$	$9,33\pm1,53^{d}$	$18,33\pm0,58^{b}$	25 ± 0^{a}	6 ± 0^{e}	6 ± 0^{e}
H	$8,00\pm0^{h,i,j}$	$8,00\pm0^{h,i,j}$	$8,67\pm0,58^{g,h,i,j}$	$18,67\pm0,58^{b}$	$24,33\pm0,58^{a}$	6 ± 0^{j}	6 ± 0^{j}
I	$12,00\pm0^{a,b}$	$10,67\pm1,15^{b}$	$11,00\pm0^{b}$	nd	nd	nd	nd
J	$11,67\pm0,58^{b}$	$7,00\pm0^{g,h}$	$11,00\pm1^{b,c}$	$24,33\pm0,58^{a}$	$25,33\pm0,58^{a}$	6 ± 0^{h}	6 ± 0^{h}
K	$11,67\pm0,58^{c}$	$9,33\pm0,58^{c,d}$	$11,67\pm0,58^{c}$	20 ± 0^{b}	31 ± 0^{a}	22 ± 0^{b}	6 ± 0^{e}

Notes: For all tables, values are Mean ±SD (n=3). Result within each strain with different letters (a - j) differs significantly ($p < 0.05$). DCM= dichloromethane; nd= not determined. **A**: *V. cholerae* (Fish); **B**: S. typhimurium (salad); **C**: *E. coli* boromo; **D**: *E. coli* (ATCC: 25922); **E**: *E. coli* (CPI: 105182); **F**: *P. mirabilis* (ATCC: 35659); **G**: *B. cerus* (ATCC: 9144); **H**: *S. aureus* (ATCC: 6538); **I**: *V. cholerae* (Water); **J**: *S. dysenteria* (CPI: 5451); **K**: *S. typhimurium* (Fish)

Table. 4: Minimum inhibitory concentration.

Fractions	CE (mg/ml)			HF (mg/ml)			DCMF (mg/ml)			EAF (mg/ml)			BF (mg/ml)		
Strains	***H. a***	***N. c***	***P. b***	***H. a***	>20	***P. b***	***H. a***	***N. c***	***P. b***	***H. a***	***N. c***	***P. b***	***H. a***	***N. c***	***P. b***
V. cholerae (Fish)	>20	>20	20	20	>20	>20	2.5	>20	5	2.5	>20	>20	2.5	>20	>20
S. typhimurium (salad)	>20	>20	20	20	1.25	2.5	>20	1.25	>20	>20	2.5	>20	0.625	>20	>20
E. coli boromo	>20	>20	>20	>20	>20	>20	>20	>20	>20	20	20	>20	>20	>20	>20
E. coli (ATCC: 25922)	>20	2.5	>20	>20	>20	>20	1.25	>20	>20	5	>20	>20	>20	>20	2.5
E. coli (CPI: 105182)	>20	>20	>20	>20	>20	10	0.3125	0.3125	0.3125	>20	>20	2.5	>20	0.3125	2.5
P. mirabilis (ATCC: 35659)	2.5	>20	5	20	>20	>20	>20	>20	>20	>20	>20	1.25	>20	0.625	>20
B. cerus (ATCC: 9144)	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
S. aureus (ATCC: 6538)	5	>20	>20	>20	>20	5	>20	>20	5	>20	20	>20	>20	>20	>20
V. cholerae (Water)	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	2.5	>20	5	>20	>20
S. dysenteria (CPI: 5451)	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
S. typhimurium (Fish)	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	20	>20	>20	>20	>20

Table. 5: Preliminary phytochemical screening.

Fraction	Plant	Positive tests for	Negative tests for
EM	**H.a**	Flavonoids, sterols/triterpenes, Saponins, tannins and polyphenols, coumarins	Anthracenosids
	N.c	Flavonoids, sterols/triterpenes Saponins, tannins and polyphenols	Coumarins, Anthracenosids
	P.b	Flavonoids, sterols/triterpenes, Saponins, tannins and polyphenols, coumarins	Anthracenosids
FH	**H.a**	sterols/triterpenes	Flavonoids, Saponins, tannins and polyphenols, coumarins, Anthracenosids
	N.c	sterols/triterpenes	Flavonoids, Saponins, tannins and polyphenols, coumarins, Anthracenosids
	P.b	sterols/triterpenes, Saponins, tannins and polyphenols	Flavonoids, coumarins, Anthracenosids
FDCM	**H.a**	Saponins	Flavonoids, sterols/triterpenes, tannins and polyphenols, coumarins, Anthracenosids
	N.c	Saponins	Flavonoids, sterols/triterpenes, tannins and polyphenols, coumarins, Anthracenosids
	P.b	Saponins, sterols/triterpenes	Flavonoids, tannins and polyphenols, coumarins, Anthracenosids
FAE	**H.a**	sterols/triterpenes, Saponins, tannins et polyphenols, Anthracenosids	Flavonoids, coumarins
	N.c	Flavonoids, Saponins, tannins and polyphenols, coumarins	sterols/triterpenes, Anthracenosids
	P.b	Saponins, coumarins	Flavonoids, sterols/triterpenes, tannins and polyphenols, Anthracenosids
FB	**H.a**	Saponins, coumarins	Flavonoids, sterols/triterpenes, tannins and polyphenols, Anthracenosids
	N.c	Saponins, coumarins, sterols/triterpenes, tannins and polyphenols,	Flavonoids, Anthracenosids
	P.b	Saponins, coumarins	Flavonoids, sterols/triterpenes, tannins and polyphenols, Anthracenosids

CE: Crude Extract; HF: Hexane Fraction; DCMF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanol Fraction; H.a: *H. auriculata*; N.c: *N. canescens*; P.b: *P. bicalyculata*

Table. 6: Polyphenols contents of extracts.

Plants	Fractions	Total phenolic(mg GAE/100 mg extract)	Total flavonoids (mg QE/100 mg extract)	Total flavonols (mg QE/100 mg extract)	Total tannins (mg TAE/100 mg extracts)
Hygrophila auriculata	CE	$80.86 \pm 0.20^{d,e}$	$12.30 \pm 0.71^{e,f,g}$	$0.65 \pm 0.03^{i,j}$	1.31 ± 0.31^{f}
	HF	80.05 ± 0.30^{e}	17.25 ± 1.20^{d}	0.56 ± 0.02^{j}	$5.79 \pm 1.29^{e,f}$
	DCMF	$81.30 \pm 0.69^{d,e}$	23.51 ± 1.05^{c}	$1.18 \pm 0.04^{f,g}$	0.73 ± 0.19^{f}
	AEF	$92.36 \pm 1.63^{a,b}$	17.30 ± 0.91^{d}	1.58 ± 0.05^{e}	48.64 ± 6.56^{b}
	BF	$89.26 \pm 1.55^{b,c}$	$16.26 \pm 0.51^{d,e}$	$0.98 \pm 0.05^{g,h}$	$8.82 \pm 2.09^{d,e,f}$
Nelsonia canescens	CE	$85.10 \pm 0.61^{c,d,e}$	$16.19 \pm 1.61^{d,e}$	$0.83 \pm 0.05^{h,i}$	$7.28 \pm 1.69^{e,f}$
	HF	$81.63 \pm 2.35^{d,e}$	25.34 ± 0.23^{c}	3.40 ± 0.02^{b}	23.64 ± 3.42^{c}
	DCMF	$84.51 \pm 0.99^{c,d,e}$	75.02 ± 3.39^{a}	8.05 ± 0.10^{a}	$17.04 \pm 1.89^{c,d}$
	AEF	$90.91 \pm 3.36^{a,b}$	26.62 ± 0.64^{c}	0.88 ± 0.09^{h}	24.37 ± 4.91^{c}
	BF	94.74 ± 3.24^{a}	44.23 ± 2.57^{b}	2.41 ± 0.09^{d}	62.52 ± 5.28^{a}
Peristrophe bicalyculata	CE	$80.24 \pm 1.51^{d,e}$	$13.18 \pm 1.61^{d,e,f}$	$0.80 \pm 0.10^{h,i}$	$7.67 \pm 2.22^{e,f}$
	HF	$80.29 \pm 0.08^{d,e}$	25.92 ± 2.48^{c}	2.85 ± 0.07^{c}	$12.98 \pm 1.24^{d,e}$
	DCMF	$85.26 \pm 0.61^{c,d}$	17.07 ± 0.77^{d}	1.35 ± 0.11^{f}	$7.82 \pm 2.70^{e,f}$
	AEF	$84.98 \pm 1.11^{c,d,e}$	08.00 ± 0.38^{g}	0.88 ± 0.04^{h}	2.04 ± 0.74^{f}
	BF	$84.85 \pm 2.02^{c,d,e}$	$08.69 \pm 0.45^{f,g}$	1.22 ± 0.08^{f}	$5.34 \pm 0.10^{e,f}$

mg GAE/100mg extracts: mg Equivalent Gallic Acid for 100mg dried extracts; mgQE/100mg extract: mg Equivalent Quercetin for 100mg dried extracts; mgTAE/100mg extract: mg Equivalent Tannic Acid for 100mg dried extracts; mmol AAE/g extract: mmol Equivalent Ascorbic Acid for 1mg dried extract; CE: crude extract; HF: hexane Fraction; DCMF: Dichloromethane Fraction; AEF: Ethyl Acetate Fraction; BF: Butanol Fraction. Result within each column with different letters (a - i) differs significantly ($p < 0.05$).

Phytochemical studies

Preliminary screening

The phytochemical constituents present in the plants samples are represented in Table 5. This preliminary phytochemical screening allowed detecting the secondary metabolites such as polyphenols, tannins, flavonoids, coumarins, saponins, anthracenosids, sterols and triterpenes. For these preliminary test carried out, *H. auriculata, N. canescens* and *P. bicalyculata* crude extracts exhibeted similarly phytochemical profiles. About fractions, a similarity as been observed in Hexane and Dichloromethane fractions for *H. auriculata* and *N. canescens* while for Butanol fraction, *H. auriculata* and *P. bicalyculata* phytochemical profile are similar.

The beneficial medicinal effects of plant materials typically result from the secondary metabolites present in the plant although and it is usually not attributed to a single compound but a combination of the metabolites (Janakiraman *et al.,* 2012). Thus, the presence of all compounds confirms the utility of our three species in traditional medicine of Burkina Faso against infectious diseases.

Polyphenols contents

Crude extracts and fractions total phenolics, total flavonoids, total flavonols and total tannins contents of the three species are shown in Table 6.

In this quantification test, *N. canescens* Butanol fraction with 94.74 ± 3.24 mg GAE/100 mg extract, its Dichloromethane fraction with 75.02 ± 3.39 mg QE/100 mg extract, its Dichloromethane fraction with 8.05 ± 0.10 mg QE/100 mg extract and its Butanol fraction with 62.52 ± 5,28 mg TAE/100 mg extracts showed the higher content respectively in total phenolics, total flavonoids, total flavonols and total tannins.

All extracts and fractions of *H. auriculata, N. canescens* and *P. bicalyculata* exhibited inhibition effect on the eleven bacteria tested in this study. This antibacterial activity could be attributed to different compounds identified in these plants. In fact, antibacterial activity is very attributed to several phytochemical such as flavonoids, saponins, tannins and terpenes (Musa *et al.*, 2008). Comparing the three plants species, the best antibacterial activity has been obtained by *N. canescens* and this result could be explained by its high phenolic contents. As indicate in the table 6, this species presented highest values of total phenolics, total flavonoids, total flavonols and total tannins. In addition, one of our study (data not shown) showed the presence of many phenolic compounds such as p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, gentisic acid, apigenin, luteolin and Quercetol. While, the antibacterial activity of a number of phenolic compounds has been demonstrate: Apigenin (Sato *et al.,* 2000), luteolin (Bashir *et al.,* 1994) and flavonols (Simin *et al.,* 2002). And, previous study demonstrated a synergy between flavonoids with antibacterial activity (Arima *et al.,* 2002). These various potentialities related to the compounds found in the extracts and fractions of our plants could explain their high use in Burkina Faso traditional medicine in the treatment of infectious diseases.

CONCLUSION

In this present study, interesting antibacterial activity has been obtained with extracts and fractions of *H. auriculata, N. canescens* and *P. bicalyculata.* This antibacterial potentiality is surely due partly to phenolic compounds contained highly in these species. *N. canescens,* a species although undocumented for its antimicrobial activities, has presented is this study a best antibacterial activity and the highest contents in total phenolics, total flavonoids, total flavonols and total tannins. The results of this study suggest that plant extracts of the three *Acanthaceae* species can be substantial source of multi-purpose bioactive principles, which act as natural antibiotics against various bacteria strains. Already now, these scientific data allow to justify the traditional use of the three plants species in Burkina Faso traditional medicine. Further molecular investigations in order to isolate the antibacterial bioactive structures need to be carried out.

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