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Research Article

In vitro Inhibition of Acetyl Cholinesterase, Lipoxygenase, Xanthine Oxidase and Antibacterial Activities of Five *Indigofera* (Fabaceae) Aqueous Acetone Extracts from Burkina Faso

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Abstract: The aim of this study is to evaluate the inhibition of oxidative stress related enzymes of aqueous acetone extracts, as well as antibacterial activity from five *Indigofera* species well-known medicinal plant from Burkina. Also are investigated in this study the potential contribution of tannins and of flavonol in these activities Particularly, aqueous acetone extracts were investigated for their Lipoxygenase (LOX), Xanthine Oxidase (XO) and Acetylcholinesterase (AChE) inhibitions that are implied in inflammation, gout and Alzheimer's etiology diseases. Interestingly, *I. macrocalyx* which had the highest flavonol content (of all) showed more inhibition against LOX and XO (51.16 and 77.33% respectively). Our study showed a significant correlation between XO inhibition and total flavonol content ($R^2 = 0.9052$). AChE was low sensible to all extracts. In contrast, the extracts were rich in tannin compounds especially in *I. tinctoria* extract. And results of the *in vitro* antibacterial activities of these extracts against five bacteria showed that all bacteria were sensible to all extracts particularly *S. typhimurium* and *B. cereus*. Our results suggest that the five studied species prove to be good sources of inhibition of the three enzymes involved in oxidative stress and also to have some antibacterial properties. That is what probably explains their uses in folk medicine, singularly, in the treatment of gout, dysentery and anti-inflammatory diseases.

Keywords: Bacterial, Burkina Faso, enzymes inhibition, flavonol, *Indigofera*, tannin

INTRODUCTION

Xanthine Oxidase (XO), Lipoxygenase (LOX) and Acetylcholinesterase (AChE) are enzymes involved in various oxidative stress diseases such as gout (Owen and Timothy, 1999) and Alzheimer's disease (Cole *et al*., 2005; Ferreira *et al*., 2006) and they are quiet implicated in several inflammatory processes (Wangensteen *et al*., 2006; Ziakas *et al*., 2006).

Moreover, there is an increasing resistance of microorganisms against available antimicrobial agents and that is of major concern among scientists and clinicians worldwide. It is generally observed that pathogenic viruses, bacteria, fungi and protozoa are more and more difficult to treat with the existing drugs (Koomen *et al*., 2002). In this current medical context, there is a constant need for new products to manage numerous infectious diseases such as tuberculoses, diarrhoeas, skin diseases, malaria as well as related oxidative stress diseases like gout, cancers, diabetes, arterioscleroses, rheumatisms, Alzheimer's and cardiovascular diseases (Halliwell and Guteride, 1990; Sweeney *et al*., 2001).

The genus *Indigofera* comprises around 700 species distributed in tropical regions. In Burkina Faso, Nigeria and India, *Indigofera colutea* (Burm.) Murril. *Indigofera macrocalyx* Guild et Perr., *Indigofera nigritana* Hook f., *Indigofera pulchra* wild. and *Indigofera tinctoria* L. have intensive popular use in the treatment of various diseases as malaria, dysentery, constipation, stomach ache, fatigue, skin disease and wounds (Abubakar *et al*., 2007; Nacoulma, 1996; Perumal Samy *et al*., 1998).

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Their antimicrobial activity (Adamu *et al*., 2005; Dahot, 1999; Esimone *et al*., 1999; Leite *et al*., 2006; Maregesi *et al*., 2008; Thangadurai *et al*., 2002), antioxidant activity (Bakasso *et al*., 2008; Gyamfi *et al*., 1999; Sreepriya *et al*., 2001), antitumor activity (Han, 1994; Rajkapoor *et al*., 2004) and anti-inflammatory activity (Aziz-Ur-Rehman *et al*., 2005; Christina *et al*., 2003; Perumal Samy *et al*., 1998; Sharif *et al*., 2005) were also shown.

This study aims to evaluate the inhibition of oxidative stress related enzymes of aqueous acetone extracts from five *Indigofera* species and their antibacterial activity as well as the contribution of tannins and flavonol in these activities.

MATERIALS AND METHODS

Chemical: Acetylcholinesterase (AChE) type VI-S from electric-eel, Acetylthiocholine Iodide (ATCI), 5,5'-Dithiobis [2-nitrobenzoic acid] (DTNB), tween 20, Bovine Serum Albumin (BSA), 15-Lipoxygenase (EC 1.13.11.12) type *I-B* (from Soybean), linoleic acid, sodium phosphate, Xanthine oxidase (EC 1.1.3.22), Xanthine and allopurinol were purchased from Sigma. Galanthamine, Sodium carbonate, aluminium trichloride ($AlCl_3$), NaCl, $MgCl_2$, boric acid, allopurinol, tannic acid, potassium phosphate and quercetin were purchased from Sigma-Aldrich, Germany and tris (hydroxymethyl aminomethane) from Aldrich Germany.

All chemicals used were of an analytical grade. Authentic standards penicillin G (1MIU) was purchased from shijiazhuang. Phama. Group. Zhangnua (China) and ampicillin sodium was from Alkem Laboratories Ltd.

Plant material: *Indigofera colutea* (Burm.) Murril., *I. macrocalyx* Guilld et Perr., *I. nigritana* Hook F., *I. pulchra* willd. and *I. tinctoria* L. were collected in the region of Ouagadougou Burkina Faso in August 2005 and identified by Pr J. Millogo, a botanist in the University of Ouagadougou. Voucher specimen numbers 01, 02, 03, 04 and 05 (respectively for *I. colutea*, *I. marcrocalyx*, *I. nigritana*, *I. pulchra* and *I. tinctoria*) were deposited in the Herbarium of Laboratoire de Biologie et Ecologie Végétales, UFR/SVT, University of Ouagadougou.

Preparation of plant extracts: For each plant, the freshly cut stems with leaves were dried at room temperature and ground to fine powder. Fifty gram of this powder was soaked in 500 mL of acetone: water (80:20 v/v) for 48 h under mechanical agitation (SM 25, Edmund BÜHLER, Germany, shaker), at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI Rotavapor R-200, Switzerland) and the remaining aqueous solutions were lyophilised using a freeze drying system (Cryodos 50, TELSTAR, Spain).

Determination of total tannin and total flavonol contents: Tannins content was evaluated by the method of European Commission (2000). Briefly, distilled water (1 mL) was mixed with extract solution (0.2 mL; 10 mg/mL), ferric ammonium citrate (0.2 mL; 3.5 mg/mL in water) and ammonium hydroxide (0.2 mL; 0.8%). The mixture was incubated in darkness at room temperature for 15 min. A blank without extract was also prepared. The absorbance was measured at 525 nm against a standard curve of tannic acid. Tannin content was expressed as mg Tannic Acid Equivalent (TAE) /100 mg of extract.

Flavonol content was estimated as described by Almaraz-Abarca *et al*. (2004). Aluminium trichloride (0.75 mL, 20% in ethanol) was mixed with the extract solution (0.75 mL, 1 mg/mL) and incubated for 10 min. Then the absorbance was read at 425 nm against a blank containing ethanol and the extract solution without aluminium chloride. Quercetin was used to produce the standard curve calibration and the results are expressed as mg Quercetin Equivalent (QE) /100 mg of extract.

Acetylcholinesterase (AChE) inhibition assay: AChE inhibiting activity was measured by the spectrophotometric method developed by Lopez *et al*. (2002) inspired from Ellman *et al*. (1961) method. Thanks to this method, the enzyme activity was determined by observing the increase of a yellow colour produced from thiocholine (resulting from acetylthiocholine hydrolysis by enzyme) when it reacts with DNTB (5, 5'-dithiobis-2-nitrobenzoic acid) ion. This can be detected at 405 nm (Rhee *et al*., 2001). Ten percent methanol in buffer was used as negative control (enzyme activity without extract), Tris-HCl buffer 50 mM, pH 8, 0.1% BSA as enzyme blanck and Galanthamine as reference standard. The substrate ATCI (Acethylthiocholine Iodide) 15 mM was prepared in water and enzyme (0.22 U/mL) in Tris-HCl buffer 50 mM, pH 8, 0.1% BSA. Kinetic reaction was followed for 3 min. The percentage of enzyme inhibition (I %) of the enzymatic reaction was determined by the following equation:

$$I\% = (E - S) / E \times 100$$

where,

E : The substrate hydrolysis kinetic by enzyme without test compound

S : The substrate hydrolysis kinetic by enzyme with test compound

Lipoxygenase inhibition assay: The inhibiting activity of Lipoxygenase was determined by the spectro photometric method described by Malterud and Rydland (2000).

The enzyme solution was obtained by preparing soybean LOX (EC 1.13.11.12) type I-B (200 U/mL) in borate buffer (167 U/mL for final concentration) with linoleic acid as substrate. Ten µL of linoleic acid, 30 µL

of ethanol and 120 mL of borate buffer (pH 9.00) were mixed to obtain the test linoleic acid solution (134 µM for final concentration). Extracts (1 mg/mL) and reference (25 µg) were prepared initially in tween-20 and diluted in borate buffer (with 0.1% of final tween-20). After incubation of the mixture at 25°C for 3 mn, kinetic was read spectrophotometrically at 234 nm for 90s.

Xanthine oxidase inhibitory activity: The XO inhibitory activity was assayed on a CECIL spectrophotometer as described by Owen and Timothy (1999) with some modifications. The assay mixture consisted of 150 µL of phosphate buffer (0.066 M, pH 7.5), 50 µL of extract solution (1 mg/mL, in phosphate buffer), 50 µL of enzyme solution (0.28 U/mL). After pre-incubation at room temperature (25°C) for 3 min, the reaction was initiated by the addition of 250 µL of substrate solution (xanthine, 0.15 M in the same buffer). A blank without enzyme solution was also prepared. The reaction was monitored for 3 min at 295 nm and velocity (V_0) was recorded. Phosphate buffer was used as negative control (activity of the enzyme without extract solution). Allopurinol was used as positive control.

The percentage of XO inhibition was calculated as the following Eq. (1):

$$I (\%) = (V_{0\ control} - V_{0\ Sample}) \times 100/V_{0\ control} \quad (1)$$

where,

$V_{0\ control}$: The activity of the enzyme in absence of the extract solution

$V_{0\ Sample}$: The activity of the enzyme in presence of the extract solution or allopurinol

Antibacterial study:

Microorganisms: The microorganisms used in this study consisted of culture collection strains: *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 13061, *Proteus mirabilis* ATCC 35659, *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC 6538. Among the five bacteria, *Bacillus cereus* and *Staphylococcus aureus* are Gram-positive bacteria; *Escherichia coli*, *Proteus mirabilis* and *Salmonella typhimurium* are Gram-negative. Before testing, pure cultures were realized with all the strains in Mueller Hinton Agar and Tryptic Soy Broth. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 Mc Ferland standard.

Antibacterial tests: The disc-diffusion assay was used to evaluate the antibacterial activity. Minimum Inhibitory Concentrations (MICs) of the extract were determined using the agar-well diffusion method. The MIC was taken as the lowest concentration of extract that caused a clear to semi-clear inhibition zone around the hole after 24 h incubation at 37°C (Cos *et al*., 2006; NCCLS, 2000). One hundred µL of each concentration (150 mg/mL) was diluted in agar solution with decreasing concentrations from 75 to 0.58 mg/mL (first well to eighth). Then, 10 µL of a microbial suspension (10^5 colony forming units, CFU/mL), were added to each well. Plates were incubated at 37°C for 24 h. MIC was considered as the lowest concentration of the extract or positive control, showing no visible growth in the well.

Sterile Petri plates (d = 54 mm) were prepared by using sterile Müller-Hinton agar (Difco) for bacterial growth. Ten µL of the bacterial suspension was used to inoculate solidified MH agar. Then sterile filter paper discs (6 mm diameter) were applied on the solidified MH and 10 µL of plant extract was applied to sterile dics. Petri plates were incubated for 24 h at 37°C. The bacterial activity was evaluated by measuring the Diameter of Inhibition Zone (DIZ) formed around the disc. All tests were performed in triplicate. The five acetone aqueous extracts obtained were Dissolved in Dimethyl-Sulfoxide (DMSO) 10% to obtain 150 mg/mL as well as benzyl penicillin and ampicillin sodium used as control positive and DMSO 10% used as control negative. Each stock solution was sterilized by filtration through a 0.2 µm Millipore sterile filter.

Statistical analysis: For statistical analysis, MS Excel software (CORREL Statistical function) was used to calculate quercetin and tannic acid equivalents, to determine inhibition percentage and to establish linear regression equations. One way ANOVA of JMP was used to determine the level of statistical significance respectively.

RESULTS AND DISCUSSION

In our previous investigation on five *Indigofera* species used in folk medicine in Burkina Faso, Polyphenols contents and antioxidant activities have been determined (Bakasso *et al*., 2008).

The present study evaluates the biological activities of five *Indigofera* species related to their enzyme inhibition, their antimicrobial activities and their composition in tannins and flavonols.

Total tannin and total Flavonol contents in the aqueous acetone extracts of the five *Indigofera* species were estimated from calibration curves ($Y = 0.0011x + 0.2236$; $R^2 = 0.9995$ for total tannin and $Y = 0.0353x + 0.0016$, $R^2 = 0.9988$ for total flavonol) and the results are consigned in Table 1. The highest total tannin content was recorded in *I. tinctoria* extract (26.63±0.12 mg TAE/100 mg) while the highest amount of flavonols was found in the extract of *I. macrocalyx* (2.93±0.07 mg QE/100 mg of dried extract). High levels of tannin were also found in *I. nigritana* and *I. colutea*. *I. pulchra* and *I. colutea* showed respectively the lowest flavonols content and tannin content.

Table 1: Phytochemical composition and enzyme inhition percentages of five *Indigofera* aqueous acetone extrats

Extracts and standards	Phytochemical composition		Enzyme inhibition percentages		
	Tannin	Flavonol	AChE	LOX	XO
I. colutea	16.42±1.03^{b}	0.46±0.02^{d}	32.24±2.10^{a}	56.61±2.83^{a}	15.19±5.25^{d}
I. macrocalyx	11.88±0.67^{c}	2.93±0.07^{a}	14.77±0.87^{b}	51.18±7.91$^{a, b}$	77.33±7.57^{a}
I. nigritana	17.30±0.66^{b}	1.00±0.06^{b}	36.06±3.67^{a}	41.11±6.12$^{b, c}$	37.45±1.43$^{b, c}$
I. pulchra	7.43±0.69^{d}	0.9±0.12$^{b, c}$	7.56±1.08^{c}	39.59±4.49^{c}	33.33±4.58^{c}
I. tinctoria	26.63±0.12^{a}	0.81±0.04^{c}	7.29±1.02^{c}	40.09±0.67^{c}	42.42±1.05^{b}
Galanthamine			52.85±1.21	-	-
Quercetin			-	52.55±2	-
Allopurinol			-	-	91.54±3.85

Acetylcholinesterase (AchE), Lipoxygenase (LOX) and Xanthine Oxidase (XO) were performed at a final concentration of 100 µg/mL; Galanthamine Hbr (25 µg/mL), Quercetin (25 µg/mL) and Allopurinol (25 µg/mL) were used as standards; Tannin and flavonol are expressed respectively as mg of tannic acid equivalent (mg TAE/100 mg) and mg of quercetin equivalent (mg QE/100 mg) for 100 mg of dried extract; Data are expressed as mean values±standard deviation (n = 3); Values within each column with different superscript letters ($^{a, b, c}$) are significantly different ($p<0.05$) as determined using ANOVA

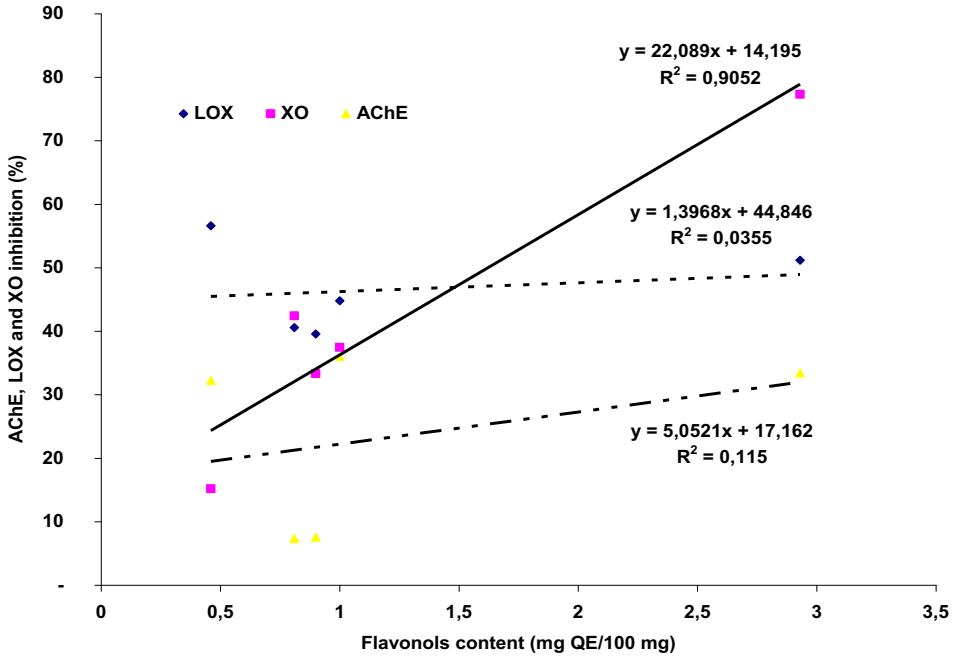

Fig. 1: Correlations between flavonol content and AChE, LOX and XO inhibition activity

The qualitative and quantitative composition in these phytochemical compounds can partially justify some of the medicinal uses of *Indigofera* species in Burkina Faso particularly to cure inflammation, skin diseases and gout.

The inhibitory activity of AChE by the five *Indigofera* species was presented in Table 1. At a final concentration of 100 µg/mL, highest inhibition was found in *I. nigritana* extract (36.06±3.67%) and *I. colutea* extract (32.24±2.10%). The lowest percent data was found in *I. pulchra* and *I. tinctoria* extracts. These results showed that *I. colutea* and *I. nigritana* which have the highest phenolic content (Bakasso *et al*., 2008) were found to possess the high inhibition of AChE. A low correlation was found between AChE inhibition and total flavonol or total tannin content (Fig. 1 and 2). Those correlations suggested that tannin and flavonol have a little contribution in AChE inhibition. However, the inhibition might come from the presence of phenolic acids, flavonoids and other antioxidant compounds. These results are in agreement with our previous investigation where we showed that *I. colutea* and *I. nigritana* had high antioxidant activity (Bakasso *et al*., 2008); consequently, antioxidant compounds might be implicated in AChE inhibition (Ferreira *et al*., 2006). Recent studies bound Alzheimer's disease to an inflammatory process induced by reactive oxygenated substances (Cole *et al*., 2005). The oxidative stress intervenes, for a share, in the physiopathology of the neuronal degeneration. AChE enzyme is considered to be related to the mechanism of memory dysfunction as Alzheimer's Disease (AD). Our extracts, particularly *I. colutea* and *I. nigritana* extracts which have the best inhibition of AChE could thus be sources of drugs to manage Alzheimer's disease; they could also have some insecticide properties since this enzyme also intervenes

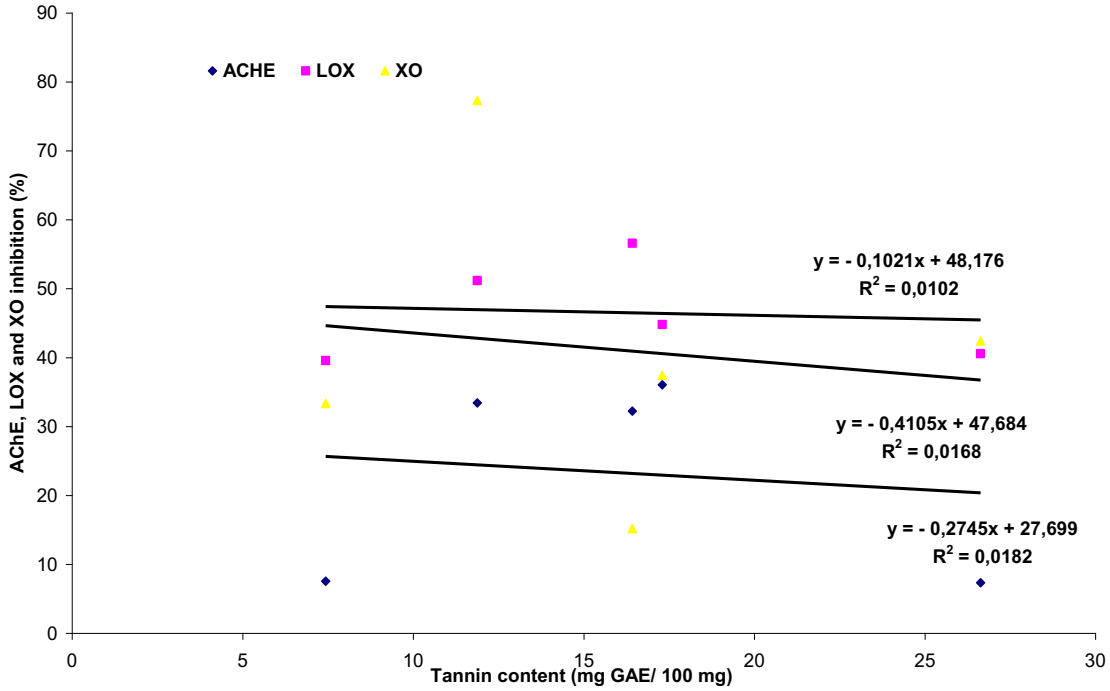

Fig. 2: Correlations between total tannin content and AChE, LOX and XO inhibition activity

in insect resistance. Galanthamine was used as standard AChE inhibitor and showed at 25 µg/mL inhibition amount 52.85%.

Lipoxygenase (LOX) is an enzyme involve in various oxidative stress disorders such as inflammation, arthritis, cancer and autoimmune diseases (Cole *et al*., 2005).

The anti-inflammatory activity of the five extracts of *Indigofera* was evaluated by measuring the inhibition of LOX using linoleic acid as substrate. The results were reported in Table 1. All of the five extracts showed inhibition percentage above 39% at 100 µg/mL. The highest inhibition was found in *I. colutea* (56.61±2.83%) and *I. macrocalyx* (51.18±9.53%) extracts. No significant differences between *I. nigritana*, *I. pulchra* and *I. tinctoria* inhibitions on LOX. The standard quercetin showed 52.55% inhibition at 25 µg/mL. It should be noted that the significant activity *I. colutea* was showing may be due to the presence of gallic acid identified in the extract of this plant (Bakasso *et al*., 2008) and recognized as made up of anti-inflammatory compounds. These results can justify the use of *I. colutea* to relieve stomachs ache (Perumal Samy *et al*., 1998). Other *Indigofera* species were previously showed to exhibit good inhibition activity against LOX (Aziz-Ur-Rehman *et al*., 2005; Sadik *et al*., 2003; Sharif *et al*., 2005).

Xanthine Oxidase (XO) activity is implicated in the etiology of gout, an inflammatory disorder characterized by the deposit of uric acid on the articulations. The production of uric acid can be blocked by inhibiting this enzyme and consequently cure the problems of gout (Chiang *et al*., 1994). XO inhibitors are known to be therapeutically useful for the treatment of gout, hepatitis and brain tumor (Filha *et al*., 2006; Song *et al*., 2003). For that, the five *Indigofera* aqueous acetone extracts were tested against XO. The results are presented in Table 1. Among the five plant extracts, four gave a data percentage of the XO inhibition superior to 33% at 100 µg/mL (final concentration). *I. macrocalyx* with 77.33±7.57% gave the best inhibition followed by *I. tinctoria* (42.42±1.05%). The lowest inhibition activity was found in *I. colutea* extract (15.19±5.25%). The best inhibition obtained with *I. macrocalyx* extract can be explained by its high flavonol content. Investigation on the XO inhibition showed that the flavonoids and, in particular, the flavonol plays a significant role in the inhibition of this enzyme (Nagao *et al*., 1999; Raj Narayana *et al*., 2001).

Previous studies showed *I. macrocalyx* methanol extract XO inhibitory activity (Bangou *et al*., 2011) This last assertion is strongly supported by the positive correlation obtained between flavonol content and XO ($R^2 = 0.9052$) (Fig. 1). The inhibiting compounds of XO have biological potentials in the treatment of gout or other diseases induced by XO activity (Kong *et al*., 2000; Sweeney *et al*., 2001). It is recognized that the inhibitors of XO are used in the treatment of hepatitis and tumours of the brain. Allopurinol used as a positive control exhibited an inhibition percentage of 91.25% on XO at 25 µg/mL.

Table 2: Antibacterial activity of five *Indigofera* aqueous acetone extracts, diameter of inhibition zone (mm)

	Gram negative bacteria			Gram positive bacteria	
Extracts	*E. coli*	*S. tiphymurium*	*P. mirabilis*	*B. cereus*	*S. aureus*
I. colutea	10±1	16±1	16±1	14±1	15±1
I. macrocalyx	13±1	15.00±2	13±1	16±1	16±2
I. nigritana	12.00±1	14.00±1	11.00±2	16±1	11±1
I. pulchra	12±2	13.00±1	10.00±0	12±1	13±1
I. tinctoria	11±1	16±1	14.00±1	15±1	14±1
Ampicillin	>53	>53	>53	R	>53
Penicillin	>53	>53	>53	R	>53

Table 3: The minimum inhibitory concentration (mg/mL) of five *Indigofera* aqueous acetone extracts

	E. coli	*S. tiphymurium*	*P. mirabilis*	*B. cereus*	*S. aureus*
I. colutea	18.75	9.37	9.37	9.37	9.37
I. macrocalyx	9.37	18.75	18.75	9.37	9.37
I. nigritana	9.37	18.75	18.75	18.75	18.75
I. pulchra	18.75	18.75	37.50	18.75	9.37
I. tinctoria	18.75	9.37	18.75	9.37	9.37

The antibacterial activity of the five *Indigofera* species was evaluated on five bacterial strains. The results of diameter of inhibition zone are reported in Table 2.

These results indicated that Ampicillin and Penicillin, two broad spectrum antibiotics, exerted strong inhibitions against four of the five tested strains (diameter >53 mm). But *Bacillus cereus* showed resistance to the two antibiotics while it displayed susceptibility of all the extracts; that could be explained by the development of enzymes such as β-lactamase by these bacteria, being able to neutralize the effect of those antibiotics.

All of the extracts express activity against the tested strains but *E. coli* was the most resistant. *I. colutea*, *I. macrocalyx* and *I. tinctoria* are more active than the other extracts on bacterial strains. The best diameters are obtained by *I. colutea* extract on *S. typhimurium* (16±1) and on *P. mirabilis* (15.67±1.15 mm). Maregesi *et al*. (2008) found that the extracts of *I. colutea* strongly inhibited the growth of *B. cereus* and *S. aureus* stains. Adamu *et al*. (2005) found that *I. pulchra* exhibited activity against *S. aureus* and *P. mirabilis*. Other investigations showed that some *Indigofera* species are active against *S. aureus*, *E. coli*, *S. typhimurium* (Esimone *et al*., 1999; Leite *et al*., 2006). In our previous investigation, *I. colutea* showed the best phenolic compounds content among the other *Indigofera* species (Bakasso *et al*., 2008). Polyphenols compounds such as phenolic acid, tannin and flavonoid are important antibacterial compounds and constitute a factor of toxicity for the micro-organisms (Scalbert, 1991). In this study, the antibacterial activity of the five *Indigofera* species can be explained by the significant correlations obtained between flavonol content and inhibition zones against *E. coli* ($R^2 = 0.6669$) (Fig. 3).

Those results also showed that in general Gram-positive bacteria are more sensitive than Gram-negative ones. Gram-negative bacteria possess an outer membrane and cell wall in comparison with the Gram-positive bacteria that could explain the weak sensibility

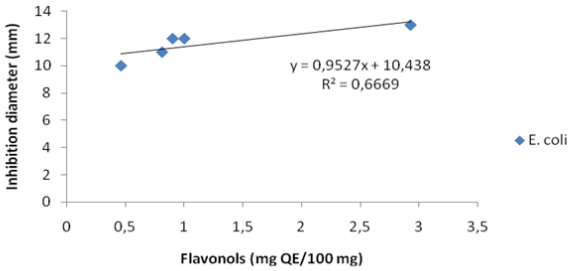

Fig. 3: Correlation between flavonol content and *E. coli* inhibition zone

of Gram-negative bacteria contrary to the Gram-positive which have not outer membrane and cell wall structure (Shan *et al*., 2007). Indeed, the mechanism of phenolic toxicity against bacteria could be through the inhibition of the hydrolytic enzymes, however, with other interactions to set of the bacterial adhesion, proteins of transport of the cell outer membrane and the non specific interaction with the carbohydrates. Phenolic compounds can also be the cause of the deprivation of iron or the hydrogen bonds of the bacterial enzymes (Scalbert, 1991).

The MIC of the different extract is consigned in Table 3. A very low MIC corresponds to a very strong antibacterial activity. These concentrations are between 9.37 and 37.5 mg/mL. *I. colutea*, *I. macrocalyx* and *I. tinctoria* showed the same activity against the various bacteria strains.

The antibacterial activity highlighted would confirm the therapeutic use of certain *Indigofera* sp., in folk medicine against skin disease and other bacterial infections (Adamou *et al*., 2005; Nacoulma, 1996).

CONCLUSION

This investigation showed that *Indigofera* species, used in folk medicine in Burkina Faso, are active on all stocks bacterial tested. In addition the enzymatic activities showed that LOX and XO are more inhibited

than AChE. Moreover, *I. macrocalyx* the richest extract in flavonol, was found to possess the strongest activity against XO. According, the plants used in this study represent a good source for XO and LOX inhibitors and also for antibacterial compounds. These results give, once again, further support to the therapeutically uses of these *Indigofera* species.

Future studies aim to isolate and identify these active constituents that exhibit significant XO and LOX inhibitory activity through bioassay-guided.

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