Antibacterial Potential of Aqueous Acetone Extracts From Five Medicinal Plants used Traditionally to Treat Infectious Diseases in Burkina Faso

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Abstract: Polyphenol contents, in vitro antioxidant and antimicrobial activities of leaves and barks from of Anogeissus leiocarpus, Khaya senegalensis, Sterculia setigera, Solanum incanum and Pterocarpus erinaceus extracts obtained with aqueous acetone 80% (WAE) were investigated in this study. The present study was conducted to evaluate the total phenolic contents, in vitro antioxidant and antibacterial properties. Folin-ciocalteu and AlCl3 methods, respectively were used for polyphenol contents. The antioxidant potential of the samples was evaluated using two separate methods, inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Iron (III) to iron (II) reduction activity (FRAP). In vitro antibacterial capacity of aqueous acetone extracts were investigated by agar disc diffusion, micro-well dilution (MIC) and Minimum Bactericidal Concentration (MBC) assay against five bacteria. Our extracts contained high levels of phenolics and exhibited antibacterial activity against bacteria. S. aureus and B. cereus were the most sensitive; while E. coli was the most resistant. There were highly positive relationships (R2 = 0.8454-0.9780) between phenolic contents and antioxidant, activity antibacterial capacity and phenolic contents of the tested extracts against each bacterium. This suggested that the antibacterial activity of the tested extracts was closely associated with their phenolic constituents. In this study, the extract of Anogeissus leiocarpus leaves was presented the best results among all extracts.

Key words: Antioxidant and antibacterial activities, polyphenol contents

INTRODUCTION

Traditional cures and plant-based remedies remain the main solution to health problems in many developing countries (Azaizeh et al., 2003). According OMS (2003), medicinal plants usefulness was estimated that over 80% of developing countries populations have resorted to traditional medicine. This need to use traditional medicine can be explained for the poverty of our populations, the lack of medical facilities and doctors, the religion and above all microbial resistance in relation to the modern medicine (Adjahnoun, 1985).

In this way, the present study concerned Anogeissus leiocarpus, Khaya senegalensis, Sterculia setigera, Solanum incanum and Pterocarpus erinaceus extracts. They are the tropical plants used in Africa particularly in Burkina Faso as a component of several primitive medicinal remedies against human and veterinary diseases. These plants have many folk uses in various African countries and it is largely used as component of many popular preparations for its antiseptic, anti-malarial, antimicrobial and anti-viral activities (Nacoulma, 1996).

Ethnobotanical investigations in the central region of Burkina Faso have showed that about Anogeissus leiocarpus, Khaya senegalensis, Sterculia setigera, Solanum incanum and Pterocarpus erinaceus extracts are used frequently and widely in traditional medicine to treat various kinds of diseases such as malaria, fever, the
treatment of human and avian gastrointestinal infections, Newcastle disease, dermatitis, varicella, variola, cardiovascular diseases, antibacterial and antiviral activities (Nacoulma, 1996), (Karou et al., 2005; Koné et al., 2004). In addition, (Nacoulma, 1996) showed that Anogeissus leiocarpus, Khaya senegalensis, Sterculia setigera, Solanum incanum and Pterocarpus erinaceus extracts, in particularly, the leaves and barks contain saponosides, cumarins, steroids and polyphenols and its may be explain the use in many diseases treatment. In this fact, the aim of the present study is to evaluate the phytochemical composition and relationships between total phenolic contents, antioxidant and antibacterial potentials from barks leaves of Anogeissus leiocarpus, Khaya senegalensis, Sterculia setigera, Solanum incanum and Pterocarpus erinaceus extracts. Antioxidant activity was evaluated using two separate methods, namely inhibition of free radical 2,2-diphenyl-1-picyrhydrazyl (DPPH) and FRAP method. In vitro antibacterial activity was determined by using agar disc diffusion, micro-well dilution (MIC) and bactericidal minimal concentration (MBC) assay with barks and leaves extracts against five bacteria for their medicinal importance in particularly, their traditional use in infectious pathologies in Burkina Faso by scientific bases.

MATERIALS AND METHODS

Chemicals and reagents: The Folin-ciocalteu reagent, NaH2PO4, Na2HPO4, sodium carbonate, aluminium trichloride, gallic acid and quercetin were purchased from Sigma-aldrich Chemie, Steinheim, Germany. 2,2-diphenyl-picyrhydrazyl (DPPH), trichloroacetic acid, and solvents used were from Fluka Chemie, Switzerland. Potassium hexacyanoferrate [K3Fe(CN)6] was from Prolabo and ascorbic acid was from Labosi, Paris, France. All chemicals used were of analytical grade. Authentic standards, such as penicillin G (1 MIU) was purchased from Shijiazhuang, Pharma. Group. Zhangnua (China) and ampicilin sodium (500 mg) was from Alkem Laboratories Ltd.

Plants material: Anogeissus leiocarpus (Combretaceae), Khaya senegalensis (Meliaceae), Sterculia setigera (Sterculiaceae), Solanum incanum (Solanaceae) and Pterocarpus erinaceus (Fabaceae) barks and leaves extracts were collected in August 2006 in Saaba, 4 Km east of Ouagadougou, Burkina Faso capital. A voucher specimen was deposited at the herbarium of University of Ouagadougou after identification by Prof. Millogo.

Bacterial strains: Five strains of bacteria from the American Type Culture Collection (ATCC, Rockville) were tested: Escherichia coli ATCC 25922, Bacillus cereus ATCC 13061, Proteus mirabilis ATCC 35659, Salmonella typhimurium ATCC 13311 and Staphylococcus aureus ATCC 6538. Among the five strains bacteria, Escherichia coli, Proteus mirabilis and Salmonella typhimurium are Gram-negative bacteria; Bacillus cereus and Staphylococcus aureus are Gram-positive bacteria.

Preparation of extracts: The collected plants material were dried at room temperature and ground to a fine powder. Fifty grams (50 g) of powdered plant material were extracted with 80% aqueous acetone (500 mL) under mechanic shaking for 48 h at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BUCHI, Rotavapor R-200, Switzerland) at approximately 40°C and freeze-dried by a being Telstar Cryodos 50 freeze-dryer. The extract residues were weighed before packed in waterproof plastic flasks and stored at 4°C until use. The yields of different crude extracts were calculated and expressed as grams of extract residues/100 g of dried plant materials.

Phytochemical analysis: The chemical analyses of aqueous acetone extracts of Anogeissus leiocarpus, Khaya senegalensis, Sterculia setigera, Solanum incanum and Pterocarpus erinaceus barks extracts were screened according the method of Ciulei (1982). That made possible to characterize the presence of secondary metabolites in plants several methods have been used. Flavonoids, tannins, saponins, coumarins, alkaloids, triterpenes and steroids and including cardenolids.

Polyphehols determination: Total phenolic contents: Total polyphenols were determined by Folin-Ciocaltceu method as described by Singleton et al. (1999). Aliquots (125 µL) of solution of extracts in methanol (10 mg/mL) were mixed with 625 µL Folin-Ciocaltceu reagent (0.2 N). After 5 min, 500 µL of aqueous Na2CO3 (75 g/L) were added and the mixture was vortexed. After 2 h of incubation in the dark at room temperature, the absorbancies were measured at 760 nm against a blank (0.5 mL Folin-Ciocaltceu reagent +1 mL Na2CO3) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (0-200 mg/L). The results were expressed as mg of gallic acid equivalents (GAE)/100 mg of extract.

Total flavonoid contents: The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grant et al. (1994). 0.5 mL of methanolic AlCl3 (2%, w/v) were mixed with 0.5 mL of methanolic extract solution (0.1 mg/mL). After 10 min, the absorbancies were measured at 415 nm against a blank (mixture of 0.5
mL methanolic extract solution and 0.5 mL methanol) and compared to quercetin calibration curve (0-200 mg/L). The data obtained were the means of three determinations. The amounts of flavonoids in plant extracts were expressed as mg of quercetin equivalents (QE)/100 mg of extract.

**Antioxidant activity determination:**

**DPPH radical method:** Radical scavenging activity of plant extracts against stable DPPH (2, 2'-diphenyl-1-picrylhydrazyl, Fluka) was determined spectrophotometrically at 517 nm as described by Velazquez et al. (2003). Extract solutions were prepared by dissolving 10 mg of dry extract in 10 mL of methanol. The samples were homogenized in an ultrasonic bath. 0.5 mL of aliquots which were prepared at different concentrations from each extract of plant was mixed with 1 mL of methanolic DPPH solution (20 mg/mL). After 15 min in the dark at room temperature, the decrease in absorption was measured. The blank sample was constituted by a same amount of methanol and DPPH solution. All experiments were performed in triplicate. Radical scavenging activity was calculated by the following formula:

\[
\text{Inhibition (\%)} = \frac{(A_0 - A/A_0)}{100}
\]

A0: absorption of blank sample
A: absorption of tested extract solution

Amount of extracts in samples and DPPH radical scavenging activity curve was plotted. The concentration which was responsible of half scavenging activity IC50 (concentration causing 50% inhibition) value of each extract was determined graphically and expressed as μg/mL.

**Iron (III) to iron (II) reduction activity (FRAP):** The FRAP assay was done according to the method of Hinnebourg et al. (2006). Briefly, 0.5 mL of each extract (1 mg/mL) was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [K3Fe(CN)6] solution (1%). After 30 min incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 2000 × g for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared FeCl3 solution (0.125 mL, 0.1%). Absorbencies were read at 700 nm and Ascorbic acid was used to produce the calibration curve. The iron (III) reducing activity determination was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of extract.

**Antibacterial activity:**

**Preparation of inocula:** The bacterial strains grown on nutrient agar at 37°C for 18-24 h were suspended in a saline solution (0.9%, w/v) to a turbidity of 0.5 Mac Farland standards (1×10^8 CFU/mL). To obtain the inocula, these suspensions were diluted 100 times in Muller Hinton broth to give 10^6 colony forming units (CFU)/mL (Ezoubeiri et al., 2005).

**Preparation of discs:** The aqueous acetone extracts obtained were dissolved in 10% aqueous dimethylsulfoxide (DMSO) (not toxic to germs at this percentage; Pujol et al., 1990) at a final concentration of 25 mg/mL and all extracts were sterilized by filtration through 0.22 μm sterilizing Millipore express filter. The sterile discs (6 mm) were impregnated with 10 μL of the sterile extracts (2 mg/disc). Negative controls were prepared using discs impregnated with 10% aqueous dimethylsulfoxide (solvent control). Commercially available antibiotic diffusion disks were used as standards for comparison. Penicillin G (1MU) from Shijiazhuang pharma. Group, Zhangnua (Chine) and Ampicillin from Alkom Laboratories LTD were used as positive reference standards (15 μg/disc) for all bacterial strains.

**Disc-diffusion assay:** Petri plates were prepared by pouring 20 mL of briefly, molten Mueller Hinton agar (DIFCO, Becton Dickinson, USA). The inoculum was spread on the top of the solidified media and allowed to dry for 10 min. The discs were then applied and the plates were left 30 min at room temperature to allow the diffusion of the extract before their incubation for 24 h at 37°C and at 44°C for *Escherichia coli* because this bacterium is thermo resistant. The inhibition zone diameters were evaluated in millimetres. The extract inducing inhibition zone diameters ≥ 3 mm around disc were considered as antibacterial. All tests were performed in triplicate (Rabe et van Stadt., 1997).

**Micro-well dilution assay:** Minimum Inhibition Concentration (MIC) was determined by the micro-dilution method in culture broth as recommended by Ellhof (1998) and the National Committee for Clinical Laboratory Standard (NCCLS, 2001). The 96-well microplates (NUNC, Danemark) containing 100 μL of Mueller Hinton (MH) broth were used. For each bacterium strain, three columns of eight wells to the micro-plates were used. Each well has getting: the culture medium + extract + inoculum (10 μL of inoculate) and INT (50 μL; 0.2 mg/mL). The micro-plates were covered and incubated overnight at 37 and at 44°C for *Escherichia coli* for 24 h. Each MIC experiment was repeated three times. Inhibition of bacterial growth was judged by rose or yellow colour. The MIC was defined as the lowest concentration at which no visible growth was observed.
Minimal Bactericidal Concentration (MBC): Minimum Bactericidal Concentration (MBC) was determined at the concentration was defined as the lowest extract concentration at which 99.9% of the bacteria population were killed after 18 or 24 h incubation overnight at 37ºC. Each experiment was repeated at least three times. The MBC is determined with the wells which are the concentration ≥ MIC according to the National Committee for Clinical Laboratory Standard (NCCLS, 2001). The MBC is determined in Mueller Hinton (MH) agar (DIFCO, Becton Dickinson, USA) medium. We used 10 µL of each well where concentration ≥ MIC on the Petri plates (Traoré et al., 1993).

Evaluation of bactericidal and bacteriostatic capacity:
The action of an antibacterial on the bacterial strain can be characterized at two parameters as Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC). According to the ratio MBC/MIC, we can apperceive antibacterial activity. If the ratio MBC/MIC=1 or 2, the effect is bactericidal but if the ratio MBC/MIC = 4 or 16, the effect is bacteriostatic (Berche et al., 1988).

RESULTS
Polyphenol contents: Our results showed that the total phenolic contents in different extracts varied greatly. The total phenolic contents ranged, respectively 70.27±3.96 mg GAE/100 mg extract for Anogeissus leiocarpus, 57.20±2.6 mgAGE/100 mg extract for Khaya senegalensis, 70.20±2.6 mgAGE/100 mg extract for Pterocarpus erinaceus, 61.30±0.44 mgAGE/100 mg extract from Sterculia setigera extract and 15.20±1.31 mg GAE/100 mg extract for Solanum incanum. The total flavonoids content ranged respectably 54.13± 0.26 mgGAE/100 mg extract for Anogeissus leiocarpus, 0.547±0.021 mgAGE/100 mg extract for Khaya senegalensis, 5.89±0.15 mgAGE/100mg extract for Pterocarpus erinaceus, 0.515±0.005 mgAGE/100 mg extract from Sterculia setigera and 0.277±0.003 mg GAE/100 mg extract for Solanum incanum. In this result, the highest values were obtained with the extract of Anogeissus leiocarpus. The lowest values were obtained with the extract of Solanum incanum. The results are respectively in the (Fig. 1).

Antioxidant activity: Two methods described gave the measures of antioxidant activity whose results were consigned in the (Fig. 2). DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Rajeshwar et al., 2005). The reduction capacity of DPPH radicals was determined by the decrease absorbance at 517 nm, which is induced by antioxidant. The values of 50% inhibition concentration (IC50) varied from 1.07±0.06 to 0.13±0.46 µg/mL. The best IC50 was found with aqueous acetone extracts of Anogeissus leiocarpus (1.07±0.06 µg/mL). The lowest value was found with the
extracts of *Solanum incanum* (0.13 ±0.46 μg/mL). The IC₅₀ for the reference compounds were 0.88±0.11 μg/mL for quercetin and 0.61±0.14 μg/mL for gallic acid. In this fact, we could say that *Anogeissus leiocarpus* has a intersect antioxidant activity compared than the reference compounds.

For FRAP assay, aqueous acetone extract of *Anogeissus leiocarpus* has also give the best antioxidant activity by FRAP method.

**Antibacterial activity:** In this activity, five bacteria used (*Escherichia coli*, *Proteus mirabilis* and *Salmonella typhimurium* ATCC 13311) were Gram-negative and two (*Bacillus cereus* ATCC 13061 and *Staphylococcus aureus* ATCC 6538) were Gram-positive. There is a significant variation in the diameters of inhibition Zones values (DIZ) of extracts (Table 1). The DIZ values for the five bacteria species were range from 14.67±0.58 to 20.67±1.53 mm for *Anogeissus leiocarpus* and 11.67±0.58 to 13.67±0.58 mm for *Solanum incanum* leaves then, 13.67±1.15 to 19±0.00 mm for *Pterocarpus erinaceus* extracts, 13.33±0.58 to 19.33±0.58 mm for *Khaya senegalensis* and 12.33±0.58 to 18.33±0.58 mm for *Sterculia setigera*. For the Gram-positive bacteria, the best antibacterial activity was obtained with aqueous acetone extract from *Anogeissus leiocarpus* from extracts against *Bacillus cereus* ATCC 25922, *Proteus mirabilis* ATCC 15609, *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC 6538. We notice that, the aqueous acetone extract from *Anogeissus leiocarpus* has a best antioxidant activity by FRAP method.

**Table 1: Antibacterial activity**

<table>
<thead>
<tr>
<th>Activities</th>
<th>Extracts/Antibiotics</th>
<th>S. aureus</th>
<th>B. cereus</th>
<th>E. coli</th>
<th>S. typhimurium</th>
<th>P. mirabilis</th>
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<tbody>
<tr>
<td>DIZ (mm)</td>
<td><em>Khaya senegalensis</em></td>
<td>19.33±0.58</td>
<td>18.67±0.58</td>
<td>13.33±0.58</td>
<td>16.33±0.58</td>
<td>14.33±0.58</td>
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<td></td>
<td><em>Sterculia setigera</em></td>
<td>16±0.00</td>
<td>18.33±0.58</td>
<td>12.33±0.58</td>
<td>15.67±0.58</td>
<td>13.33±0.58</td>
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<td></td>
<td><em>Pterocarpus erinaceus</em></td>
<td>18.67±0.58</td>
<td>19±0.00</td>
<td>13.67±1.15</td>
<td>16.67±0.58</td>
<td>14.67±0.58</td>
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<td></td>
<td><em>Anogeissus leiocarpus</em></td>
<td>20.67±1.53</td>
<td>19.21±1.15</td>
<td>14.67±0.58</td>
<td>17.33±0.58</td>
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<td><em>Solanum incanum</em></td>
<td>13.67±0.58</td>
<td>13.33±0.58</td>
<td>10.33±0.58</td>
<td>11.67±0.58</td>
<td>12.33±0.58</td>
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<tr>
<td>Penicillin G</td>
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<td>R</td>
<td>&gt;53</td>
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<td>Ampicillin</td>
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<tr>
<td>MIC (μg/mL)</td>
<td><em>Khaya senegalensis</em></td>
<td>390</td>
<td>390</td>
<td>1560</td>
<td>781</td>
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<td><em>Sterculia setigera</em></td>
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<td>781</td>
<td>3125</td>
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<td></td>
<td><em>Pterocarpus erinaceus</em></td>
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<td>390</td>
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<td>MBC (μg/mL)</td>
<td><em>Khaya senegalensis</em></td>
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DIZ includes diameter of discs (6 mm); -: No inhibitory activity against this bacterium; R: resistant; Nd: Not determined.
Fig. 3: Correlation between total phenolics and AOA

note that the extracts showed an interesting activity against *Bacillus cereus* and *Staphylococcus aureus* growth comparatively the antibiotics which do not have effect on these bacteria.

The bactericidal and bacteriostatic effect of our different extracts was determined using the ratio MBC/MIC (Table 1). In general, this effect, for Gram-positive bacteria was 2 for *Anogeissus leiocarpus*. For Gram-negative bacteria such as *Salmonella typhimurium* and *Proteus mirabilis*, aqueous acetone extract of *Anogeissus leiocarpus* giving 2 as value. But for *Escherichia coli*, the value was 4 for the different extracts. Also, we remark that, for the different bacteria species, *Solanum incanum* and *Sterculia setigera* gave 4 as value. In conclusion, for our different extracts, Gram-positive bacteria were generally more sensitive to the tested extracts than Gram-negative bacteria. *S. aureus* and *B. cereus* were the most sensitive; while *E. coli* was the most resistant. *Anogeissus leiocarpus* gave the best antibacterial activity.

**Relationships between total phenolics, antioxidant and antibacterial activities:** The correlations between total phenolics, antioxidant capacity and antibacterial activity are shown (Fig. 3). The R²-values were between 0.9436 and 0.9723. The relationships between total phenolics and antibacterial activity of the different extracts were computed (Fig. 4). The R²-values were 0. 8454 and 0.978.

**DISCUSSION**

In generally, our results were interesting and above all with aqueous acetone extract of *Anogeissus leiocarpus*. The results of polyphenol contents could be explained by the fact that, acetone is the best extraction solvent of polyphenol compounds. In addition, total phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators (Cakir
et al., 2003). In this fact, we should say that phenolic compounds can contribute to the best Iron (III) to iron (II) reduction activity (FRAP) (Miliauskas et al., 2004). Then, we can say that the antioxidant capacity shows that total phenolics are responsible to the antioxidant capacity; that is to say that there is a correlation between total phenolics and the antioxidant capacity (Miliauskas et al., 2004). That is demonstrating by our results.

For antibacterial activity, we remark that our extracts showed relatively the best inhibitory activity against the Gram-positive bacteria comparatively to the Gram-negative bacteria. Also, the aqueous acetone extracts gave the best antioxidant activity, because acetone is a good solvent to extract polyphenol contents. So, this study has shown a link between the concentration of phenolic compounds in the extracts and their antibacterial activity. Among the five bacteria tested, S. aureus and B. cereus were the most sensitive to our extracts, while E. coli was the most resistant. The highest sensitivity of S. aureus and B. cereus may be due to its cell wall structure and outer membrane (Zaika, 1988). Our results suggest that Gram-positive bacteria are generally more sensitive in the extracts than Gram-negative bacteria (Lopez et al., 2005). A possible explanation for these observations may lie in the significant differences in the outer layers of Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and unique periplasme space not found in Gram-positive bacteria (Nikaido et al., 1996). The resistance of Gram-negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules and is also associated with the enzymes in periplasme space, which are capable of breaking down the molecules introduced from outside (Gao et al., 1999). Gram-positive bacteria do not have such an outer membrane and cell wall structure. Phenolic and terpenic antimicrobial activities are well documented (Rabe et al., 2002). Polyphenols, such as tannins and flavonoids, are important antibacterial activity (Machado et al., 2002). Also, polyphenols have a good antimicrobial activity against the biggest number of bacterial as such Escherichia coli, Proteus mirabilis, Salmonella typhimurium, Bacillus cereus and Staphylococcus aureus (Scalbert, 1991). These results show the good antibacterial activity with our aqueous acetone extracts, above all aqueous acetone extracts of Anogeissus leiocarpus, because acetone 80% is the best extraction solvent from polyphenols. So, which is an explanation of our results that is to say the fact that the aqueous acetone extracts which rich in polyphenol compounds which have a good antibacterial activity. On the other part, the Gram-positive bacteria are resistant on the antibiotics (Penicillin G and ampicillin). That can explain by the fact that S. aureus and B. cereus may be some β-lactamase (penillinas) which inhibit antibiotics actions (Li et al., 2007).

The mechanism of toxicity of polyphenol against microbial could be explained by the hydrolytic (proteases and carboxydrases) or to the other interactions whose can destroy microbial andesine (enzyme), sometimes the transport proteins (Cowen, 1999). In addition tannins can realise some polymerisations during oxidation reactions and this could have a toxicity effect again microbial strains (Gibbons, 2003; Scalbert, 1991).

CONCLUSION

In conclusion, we could keep that there are a correlation between total phenolics, antioxidant and antibacterial activities. Our results corroborate with Shan et al. (2007) results, because R² values were between 0.8454 and 0.9780 that is to say nearby to 1.00. The results of different correlations were presented by (Fig. 3 and 4). In this fact, these results confirm indeed the traditional use of our plants in infectious pathologies in Burkina Faso.

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