

## PHYTOCONSTITUENTS ANALYSIS, ANTIOXIDANT CAPACITY AND ANTIMICROBIAL PROPERTIES OF EXTRACTS FROM *LAGGERA AURITA* L. (ASTERACEAE)

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### ABSTRACT

**Objective:** This research was designed to evaluate the polyphenolic content, antibacterial potency and antioxidant activity of extract and fractions from *Laggera aurita* L. (Asteraceae), a medicinal herbaceous from Burkina Faso.

**Methods:** Folin ciocalteu and AlCl<sub>3</sub> methods respectively were used for polyphenol contents. The antioxidant activities of the samples were evaluated by various in vitro assays like ferrous reducing antioxidant assay (FRAP), Total reducing power, 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ABTS radical cation decolorization assays. *In vitro* antibacterial capacity of bioactive fractions were investigated by agar disc diffusion, micro-well dilution (MIC), Minimum Bactericidal Concentration (MBC) assay and at least the effect of the best bioactive fraction (EAF) from *Laggera aurita* L. (Asteraceae) and in combination with gentamycin against food bacterial strains multi-resistant was evaluated.

**Results:** Estimation of Total phenolic and flavonoids contents revealed that EAF and DCMF have the highest phenolic and flavonoid contents 62.12±0.68mgGAE and 10.56±0.29mgQE respectively. These results indicated that most of the bioactive fractions from *Laggera aurita* L. were able to inhibit Gram-positive bacteria as compared to Gram-negative bacteria. Among the samples tested for antioxidant activities, EAF and DCMF have the highest activities compared to other fractions.

**Conclusion:** These findings suggested that *Laggera aurita* L. is not only an important source for antibacterial component, but also a potential source of antioxidants. So, these results may be useful in explaining the medicinal applications of *Laggera aurita* L.

**Keywords:** *Laggera aurita* L., Bioactive fractions, Polyphenol compounds, Antioxidant and Antimicrobial properties.

### INTRODUCTION

The use of plants in the management and treatment of diseases started with life. It has been observed that many plants do indeed have medicinal value and extracts from these plants have been used to make modern drugs [1]. According to WHO, more than 80% of the world's population relies on plant based herbal medicines for their primary health care needs [2]. Also, Plants contain phytochemicals with various bioactivities, including, antioxidant, anti-inflammatory and anticancer activities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines [3].

In effect, indigenous plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties. In recent years, there is an increase in drug resistant strains of human pathogenic bacteria all over the world. One way to prevent antibiotic resistance of pathogen species is to use new compounds that are not based on existing synthetic antimicrobial agents [4]. Systematic screening of plants may result in the discovery of novel effective compounds which would tackle the problem of drug resistance.

The overproduction of reactive oxygen species like hydroxyl radical, superoxide anion radical, hydrogen peroxide radical can contribute to oxidative stress [5]. Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including diabetes, hypertension, coronary heart disease, cancer etc [6]. Most of the reactive oxygen species are scavenged by endogenous defense systems. But these systems may not be completely efficient requiring them to depend on exogenous antioxidants from natural sources. Presently, there has been an amplified interest worldwide to identify antioxidant compounds which are pharmacologically effective or have low or no side effects for use in preventive medicine and the food industry [7]. Generally antioxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicine [8].

*Laggera aurita* L. belongs to the family of Asteraceae is an annual herbaceous which found growing as weeds in Nigeria and spread throughout the sub-Saharan Africa [9]. In Burkina Faso, ethnobotanical investigations in the central region of Burkina Faso have shown that this Lamiaceae is used frequently and widely in traditional medicine to treat various kinds of diseases such as malaria, fever, pain, stomatitis, astmatic, bronchitis and nasal congestion and has also antibacterial activity [10]. Although this specie has been reported to be of significant ethnomedicinal use in Asia country like China, there is little or no ethno-use reported in Africa, except in Cameroon where it has been reported for use in cereal grains preservation and in Nigeria as remedy for pediatric malaria [11, 12]. The Asian researchers have also done a lot of work and have reported various bioactive properties of these plants [12]. Anti-inflammatory, antiviral, antibacterial and hepatoprotective properties have been reported in literature [13, 14, 15]. Phytochemical analysis showed the presence of a number of sesquiterpenoids, monoterpenoids and some flavonoids with some bioactivity have been isolated [16, 17]. However, no report has been made on the possible correlation study between polyphenol compounds, free radical scavenging capacity and antibacterial of extracts from *Laggera aurita* L. (Asteraceae). Considering the above, this work was aimed at studying the possible correlation study between polyphenol compounds, free radical scavenging capacity and antibacterial of extracts from *Laggera aurita* L. (Asteraceae).

### MATERIALS AND METHODS

#### Materials

#### Chemicals and reagents

The Folin-ciocalteu reagent, NaH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, sodium carbonate, aluminium trichloride, gallic acid and quercetin were purchased from Sigma-aldrich chemie, Steinheim, Germany.

2,2-diphenylpicrylhydrazyl (DPPH), trichloroacetic acid, and solvents used were from Fluka Chemie, Switzerland. Potassium hexacyanoferrate [K<sub>3</sub>Fe(CN)<sub>6</sub>] was from Prolabo and ascorbic acid was from Labosi, Paris, France. All chemicals used were of analytical grade. Authentic standard, such as Gentamycin (25 µg) was purchased from Alkom Laboratories LTD. INT (p-iodonitrotetrazolium chloride) was purchased from sigma-Aldrich chemie (Steinheim, Germany).

#### Plant materials

*Laggera aurita* L. (Astereceae) was collected in September 2011 in Gampela, 25 Km east of Ouagadougou, capital of Burkina Faso. The plant was identified in the Laboratory of Biology and Ecology, University of Ouagadougou, where a voucher specimen was deposited.

#### Bacterial strains and antibiotics

Ten references of food bacterial strains (Gram-positive and Gram-negative) were tested: Kd = *Bacillus cereus* MADM 1561, Bc = *Bacillus cereus* MADM 1291, Lm1 = *Listeria monocytogenes* 057, Lm 2 = *Listeria monocytogenes* Scott A, S. inf = *Salmonelle infantis* SKN 557, S.oro = *Salmonella oranienburg* SKN 1157, S.nig = *Salmonella Nigeria* SKN 1160, S.typ = *Salmonella typhimurium* SKN 533, Ec = *Escherichia coli* 81 nr. 148 SKN 541, Ye = *Yersinia enterocolitica* 6A28 SKN 599. All bacterial strains come from Culture Collection of Department of Food Science, Food Microbiology in Copenhagen University, Denmark [18].

#### Methods

##### Preparation of extract

The collected plants materials were dried at room temperature and crushed into a fine powder. Fifty grams (50g) of powdered plant material were extracted with 80% aqueous ethanol (500 ml) in ratio 1/10 (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, ethanol was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R-200, Switzzeland) at approximately 40°C and freeze-dried by Telstar Cryodos 50 freeze-dryer. These ones were filtered and freeze-dried. The extract residues were weighed before packed in waterproof plastic flasks and stored at 4°C until use. The yields of different crude extract were calculated and expressed as grams of extract residues/100 g of dried plant materials.

##### Fractionation

Fifty grams (50g) of powdered plant material were extracted with 80% aqueous ethanol (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, ethanol was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R-200, Switzzeland) at approximately 40°C. The aqueous extracts were subjected to sequential liquid-liquid extraction with oil ether, dichloromethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain oil ether fraction (OEF), dichloromethane fraction (DCMF), ethyl acetate fraction (EAF) and n-butanol fraction (n-BF). The different fractions were freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4°C until use.

##### Polyphenols determination of extract and fractions from *Laggera aurita* L. (Astereceae)

###### Total phenolic content

Total polyphenols were determined by Folin-Ciocalteu method as described by [19]. Aliquots (125 µl) of solution from extract or each fraction in methanol (10 mg/ml) were mixed with 625 µl Folin-Ciocalteu reagent (0.2 N). After 5 min, 500 µl of aqueous Na<sub>2</sub>CO<sub>3</sub> (75 g/l) were added and the mixture was vortexed. After 2 h of incubation in the dark at room temperature, the absorbencies were measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu reagent + 1 ml Na<sub>2</sub>CO<sub>3</sub>) 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 (Y = 0.0289x - 0.0036; R<sup>2</sup> = 0.9998). The results were expressed as mg of gallic acid equivalents (GAE)/100 mg of extract or fractions.

###### Total flavonoid content

The total flavonoids were estimated according to the Dowd method as adapted by [19]. 0.5 ml of methanolic AlCl<sub>3</sub> (2%, w/v) were mixed with 0.5 ml of extract or each fraction solution (0.1 mg/ml). After 10 min, the absorbencies were measured at 415 nm against a blank (mixture of 0.5 ml extract solutions and 0.5 ml methanol) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) and compared to a quercetin calibration curve (Y = 0.0289x - 0.0036; R<sup>2</sup> = 0.9998). 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 or fractions.

##### In vitro antioxidant activity of extract and fractions from *Laggera aurita* L. (Astereceae)

###### DPPH radical method

Radical scavenging activity of extract or each fraction against stable DPPH (2, 2'-diphenyl-1-picrylhydrazyl, Fluka) was determined with a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) at 517 nm as described by [19]. 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 sample of extract 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. All experiments were performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of extract or fraction (Y = -16.815x + 6.8373; R<sup>2</sup> = 0.9976). Quercetin was used as positive control.

###### ABTS radical cation decolorization assay

For ABTS radical cation decolorization assay, the procedure followed the method of [19]. ABTS was dissolved in water to a 7mM concentration. ABTS radical cation (ABTS<sup>+</sup>) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 h before use. This mixture was diluted with ethanol to give an absorbency of 0.7 ± 0.02 units at 734 nm using a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). For our study, we used 10 µL of the diluted sample (1 mgmL<sup>-1</sup> in methanol) which was allowed to react with 990 µL of fresh ABTS<sup>+</sup> solution and the absorbance was taken 6 min exactly after initial mixing. Ascorbic acid was used as standard (Y = -0.0342x + 0.634; R<sup>2</sup> = 0.9996) and the capacity of free radical scavenging was expressed as mmol Ascorbic Acid Equivalent per g of extract or fraction. Quercetin, a reference compound was used as positive control.

###### Iron (III) to iron (II) reduction activity (FRAP)

The FRAP assay was performed according to [20]. 0.5 mL of extract or each fraction (1 mgmL<sup>-1</sup>) was mixed with 1.25 mL of phosphate buffer (0.2M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [K<sub>3</sub>Fe(CN)<sub>6</sub>] 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 (0.625mL) and a freshly prepared FeCl<sub>3</sub> solution (0.125mL, 0.1%). Absorbencies were read at 700 nm on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) and Ascorbic acid was used to produce the calibration curve (Y = 0.008x - 0.0081; R<sup>2</sup> = 0.9999). The iron (III) reducing activity determination was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of extract or fractions. Troloc, a reference compound was used as positive control.

##### In vitro antibacterial activity of the bioactive fractions (DCMF and EAF) from *Laggera aurita* L. (Astereceae)

###### Preparation of inocula

The susceptibility tests were performed by Mueller Hinton agar-well diffusion method [21]. The bacterial strains grown on nutrient agar

at 37 °C for 18 h were suspended in a saline solution (0.9 %, w/v) NaCl and adjusted to a turbidity of 0.5 Mac Farland standard (10<sup>8</sup> CFU/ml). To obtain the inocula, these suspensions were diluted 100 times in Muller Hinton broth to give 10<sup>6</sup> colony forming units (CFU)/ml.

#### Preparation of discs

The stock solutions of extract or each fraction were dissolved in 10 % dimethylsulfoxide (DMSO) in water at a final concentration of 10 mg/ml. The stock solutions of 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 solution of extract or each fraction. Negative controls were prepared using discs impregnated with 10 % DMSO in water and commercially available antibiotic diffusion discs (gentamycin 25 µg from Alkom Laboratories LTD) were used as positive reference standards for all bacterial strains [21].

#### Disc-diffusion assay

Petri plates (9 cm) were prepared with 20 ml of a base layer of molten Mueller Hinton agar (DIFCO, Becton Dickinson, USA). Each Petri plate was inoculated with 15 µl of each bacterial suspension (10<sup>6</sup> CFU/ml). After drying in a sterile hood, 6 mm diameter discs soaked with 10 µl of the different extract solution dilutions were placed on the agar. Discs containing gentamycin (25 µg) were used as positive controls and 10 % DMSO was used as a negative control. The plates were incubated for 24 h at 37 °C. The diameters of the inhibition zones were evaluated in millimeters. The extract inducing inhibition zone ≥ 3 mm around disc were considered as antibacterial. All tests were performed in triplicate and the bacterial activity was expressed as the mean of inhibition diameters (mm) produced [21].

#### Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by the microdilution method in culture broth as recommended by [21, 22]. Eight serial two-fold dilutions of extracts or conventional antibiotic (gentamycin) were prepared as described before, to obtain final concentration range of 1000 µg/ml to 15.625 µg/ml. The last wells (n<sup>o</sup>8) served as sterility controls (contained broth only) or negative control (broth + inoculums). The 96-well micro-plates (NUNC, Denmark) containing 100 µL of Mueller Hinton (MH) broth were used. For each bacteria strain, three columns of eight wells to the micro-plate were used. Each well has getting: the culture medium + extracts or gentamycin + inoculum (10 µl of inocula) and INT (50 µl; 0.2 mg/ml for 30 min). The plates were covered and incubated at 37°C for 24 h. All tests were performed in triplicate and the bacterial activity was expressed as the mean of inhibitions produced. Viable microorganisms reduced the yellow dye to a pink colour. The MIC was defined as the lowest concentration of substance of extracts at which no colony was observed after incubation. So, the MIC was defined as the lowest concentration where no change was observed, indicating no growth of microorganism.

#### Minimal bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) was recorded as a lowest extract concentration killing 99.9% of the bacterial inocula after 24 h incubation at 37°C. Each experiment was repeated at least three times. MBC values were determined by removing 100 µl of bacterial suspension from subculture demonstrating no visible growth and inoculating nutrient agar plates. Plates were incubated at 37°C for a total period of 24 h. The MBC is determined with the wells whose the concentrations are ≥ MIC [21, 23]. The MBC were determined in Mueller Hinton (MH) agar (DIFCO, Becton Dickinson, USA) medium.

#### Evaluation of bactericidal and bacteriostatic capacity

The action of an antibacterial on the bacterial strains can be characterized with two parameters such as Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). According to the ratio MBC/MIC, we appreciated antibacterial activity. If the ratio MBC/MIC = 1 or 2, the effect was considered as bactericidal but if the ratio MBC/MIC = 4 or 16, the effect was defined as bacteriostatic [23].

#### Evaluation of the fractional inhibitory concentration index of the best bioactive fraction (ethyl acetate rich-flavonoid fractions) in combination with gentamycin against food bacterial strains multi-resistants

The Muller Hinton agar dilution method was used to evaluate the Fractional Inhibitory Concentration Index (FICI) of flavonoid-rich fractions from *Laggera aurita* L. (Astereceae) and the tested antimicrobial standards as reported earlier [21, 22]. Eight serial two-fold dilutions of flavonoid-rich fractions were prepared as described before, to obtain final concentration range of 1000 µg/ml to 15.625 µg/ml. A series of two-fold serial dilutions of gentamycin was also prepared in the same conditions as flavonoid-rich fractions. In this way, all antibacterial standards dilutions were mixed with the appropriate concentration of flavonoid-rich fractions thus obtaining a series of the combinations of conventional antibiotics and flavonoid-rich fractions. The concentrations prepared corresponded to 1;1/2; 1/4; 1/8; 1/16; 1/32; 1/64; of MIC values. The 96-well micro-plate (NUNC, Denmark) containing 100µL of Mueller Hinton (MH) broth were used. For each bacteria strain, three columns of eight wells to the micro-plate were used. Each well has getting: the culture medium + combination of flavonoid-rich fractions with gentamycin + inoculum (10 µl of inocula) and INT (50 µl; 0.2 mg/ml for 30 min). The plates were covered and incubated at 37 °C for 24 h. All tests were performed in triplicate and the bacterial activity was expressed as the mean of inhibitions produced. Viable microorganisms reduced the yellow dye to a pink colour. Inhibition of bacterial growth was judged by rose or yellow colour. The analysis of the combination of flavonoid-rich fractions and gentamycin was obtained by calculating the Fractional Inhibitory Concentration Index (FICI) as follows: FICI = (MIC of the combination of flavonoid-rich fractions with gentamycin /MICa alone) + (MIC of the combination of flavonoid-rich fractions with gentamycin /MICb alone), where MICa (Minimal Inhibitory Concentration of flavonoid-rich fractions from *Laggera aurita* L. and MICb (Minimal Inhibitory Concentration of gentamycin). The FICI was interpreted as follows: (1) a synergistic effect when FICI < 0.5; (2) an additive or indifferent effect when FICI > 0.5 and < 1 and (3) an antagonistic effect when FICI > 1. This study was carried out following [24].

#### Statistical analysis

The data were expressed as Mean ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at p < 0.05 and linear regression) was carried out with XLSTAT 7.1.

#### RESULTS

##### Polyphenol content

The total phenolics content per 100 mg of *laggera aurita* L. extract and fractions ranged from 62.12 ± 0.68 mgGAE to 9.07 ± 0.17 mgGAE. The highest content of total phenolics was detected in EAF with 62.12 ± 0.68 mgGAE following by DCMF with 23.67 ± 0.05 mgGAE. The lowest total phenolics were obtained in OEF with respectively 9.07 ± 0.17 mgGAE.

The total flavonoids content per 100 mg of *Laggera aurita* L. extract and fractions ranged from 10.56 ± 0.29 mgQE to 1.28 ± 0.20 mgQE. The highest content of total flavonoids in *Laggera aurita* L. was detected in DCMF with 10.56 ± 0.29 mgQE following by EAF with 10.12 ± 0.06 mgQE. The lowest total flavonoids were obtained in OEF with respectively 1.28 ± 0.14 mgQE. The results are recorded in the (Figure 1)

##### Antioxidant activity

The measures of antioxidant activity were obtained using three described methods. Results are consigned in the (Figure 2). The reduction capacity of DPPH radicals was determined by the decrease of the absorbance induced by antioxidant at 517 nm, which is induced by antioxidant. The values of different concentrations varied respectively from (9.472 ± 0.1941 mmol AAE/g extract to 4.907 ± 0.0587 mmol AAE/g extract). From these result, the strongest DPPH activity was obtained by EAF with 9.472 ± 0.1941

mmol AAE/g extract followed by DCMF with  $9.347 \pm 0.0602$  mmol AAE/g extract. The lowest activity was obtained by OEF with  $4.907 \pm 0.0587$  mmol AAE/g extract. Control compound gave  $13.76 \pm 0.26$  mmol AAE/g extract for Quercetin.

For ABTS assay, the following were values obtained respectively  $0.78 \pm 0.083$  mmol AAE/g extract for EHA,  $0.58 \pm 0.04$  mmol AAE/g extract for OEF,  $2.03 \pm 0.308$  mmol AAE/g extract for DCMF,  $2.9 \pm 0.003$  mgAGE/100mg extracts for EAF and  $1.85 \pm 1.43$  GAE/100 mg extract for BF. From these result, the strongest ABTS activity was obtained by EAF with  $2.9 \pm 0.003$  mmol AAE/g extract followed by DCMF with  $2.03 \pm 0.308$  mmol AAE/g extract. The lowest activity was obtained by OEF with  $0.58 \pm 0.04$  mg GAE/100 mg extract. The reference compound is Quercetin  $7.81 \pm 0.21$  mmol AAE/g extract.

For FRAP radical cation decolorization assay, the different values were respectively,  $1.22 \pm 0.057$  mmol AAE/g extract for EHA,  $1.73 \pm 0.026$  mmol AAE/g extract for OEF,  $5.99 \pm 0.029$  mgGAE/100mg extracts for DCM,  $3.72 \pm 0.182$  mgGAE/100mg extracts for EAF,  $3.39 \pm 0.108$  mg GAE/100 mg extract for BF. From these result, the strongest FRAP activity was obtained by DCMF with  $5.99 \pm 0.029$  mmol AAE/g extract followed by EAF with  $3.72 \pm 0.182$  mgGAE/100mg extracts. The lowest activity was obtained by OEF with  $0.67 \pm 0.06$  mg GAE/100 mg extract. Control compound gave  $7.46 \pm 3.38$  mmol AAE/g extract for Trolox.

### Antibacterial capacity

In this present study, ten bacteria strain (Gram-negative and Gram-positive bacteria) were used. The antibacterial assays were performed by the broth micro dilution methods; so that they could be qualified and quantified by inhibition zone diameters, MIC and MBC and Fractional Inhibitory Concentration Index (FICI). One noticed that the susceptibility of the bacteria to the polyphenol compounds alone and in combination with conventional antibiotic (gentamycin) varied according to microorganism. As for the micro-well dilution assay (MIC) and Minimum bactericidal concentration (MBC) of polyphenol compounds alone and in combination, result varied according to the microorganism (Table 1 and Table 2). The MIC values were ranged from  $500 \mu\text{g/ml}$  to  $31.25 \mu\text{g/ml}$  and for the MBC values were ranged from  $1000 \mu\text{g/ml}$  to  $62.5 \mu\text{g/ml}$ . The bactericidal and bacteriostatic effect of polyphenol compounds alone and their combination with conventional antibiotics was determined using the ratio MBC/MIC (Table 3). At last, with regard to FICI, our results indicate synergistic and additive effects between polyphenol compounds (flavonoid-rich fractions) and the conventional antibacterial (Table 4). One noticed that ethyl acetate fraction presented the best antibacterial activity than gentamycin.

### DISCUSSION

Medicinal plants are an important source of antioxidants [25] and natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke [26]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark [27]. There are many synthetic antioxidants in use. It is reported, however, they have several side effects, such risk of liver damage and carcinogenesis [28]. There is a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.

The phytochemical screening revealed in this research the presence of polyphenol content, steroids and triterpenes. The presence of the metabolites in our extracts has already been reported by [10]. The results showed that EAF following DCMF presented the highest amount of polyphenol content than the other fractions. The abundance of these fractions in polyphenols may explain the traditionally use of this Asteraceae in the treatment of bacterial infections. Because polyphenols are important antibacterial activity [29]. The abundance of two fraction extracts in polyphenol content should also explained antioxidant activity results. In effect, it is well known that, total phenolics constitute one of the major groups of compounds antioxidants [30]. Also, Phenols are very important

plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidative action [31]. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables [32]. Phenolic compounds from plants are known to be good natural antioxidants. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [33].

About the antioxidants properties, three methods were used for a best appreciation of our results; because a recent study demonstrates that there are differences between the test systems for the determination of the antioxidants properties [34, 35]. As a matter of fact, it is interesting to evaluate at last two or more methods. Analysis of our results shows a relationship between polyphenol content and antioxidants properties as [36, 37, 38] showed linear. These findings are in accordance with the earlier reports on total phenolic and antioxidant activity in relationship extracts [39].

Moreover, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. They provide a good source of anti-infective agents, for example emetine, quinine and berberine which still remain to be highly effective instruments in the fight against microbial infections. Various publications have documented the antimicrobial activity of plant extracts [40, 41, 42]. The results obtained in this study indicate a considerable difference in antibacterial activity with extracts. The bacteriostatic and bactericidal activity could be ascribed to the presence of polyphenolic compounds. In effect, some previous studies showed that polyphenolic compounds cause inhibition of a wide range of microorganisms. Phenol is well known as a chemical antiseptic [43]. In addition, Phenolic and terpenic antimicrobial activities are well documented [44]. Polyphenols, such as tannins and flavonoids, are important antibacterial activity [45]. The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins [46].

The results indicated that most of the extracts were able to inhibit Gram-positive bacteria as compared to Gram-negative bacteria. This is further confirmed by the previous studies by [47, 48] that describe the high sensibility of Gram-positive bacteria towards plant extracts and their component. Certain authors as [49] reported that Gram-negative bacteria are more resistant to the plant-based organic extracts because the hydrophilic cell wall structure of Gram-negative is constituted essentially of a lipopolysaccharide (LPS) that blocks the penetration of hydrophobic oil and avoids the accumulation of organic extracts in target cell membrane [50]. This is the reason why Gram positive bacteria were found to be more sensitive to various extracts.

One notice that extracts are more sensible on certain bacteria strains than standard drug (gentamycin). According a study [51], a probable degree of lipophilicity might be responsible for the extracts being higher in activity than standard drugs used lipophilicity toxicity is due to the interactions with the membrane constituents and their arrangement. Considering the above, Gram-positive bacteria should be more susceptible since they have only an outer peptidoclycans layer which is not an effective permeability barrier as reported by [52]. But in this study, we found contradicting results. *Bacillus cereus* some Gram-positive has developed resistance to the  $\beta$ -lactam antibiotics due to the production of chromosomal or plasmid mediated  $\beta$ -lactamases or by producing penicillin binding proteins (PBPs). All the *Staphylococcus aureus* strains have from PBPs (PBP1 to PBP4), but MRSA express a special PBP (PBP2 or PBP2a) from the mec A gene PBP2a takes over the biosynthetic function of normal PBPs in the presence of inhibitory concentration of  $\beta$ -lactams because PBP2 has a decreased binding affinity to  $\beta$ -lactams [53]. This has resulted in the development of multidrug resistance against  $\beta$ -lactam and other antibiotics. In addition, the polysaccharide capsular material in some of the pathogenic microorganism is responsible for virulence and antimicrobial resistance [54].

**Table 1: Minimum Inhibitory Concentration ( $\mu\text{g/ml}$ ) of bioactive fractions (DCMF and EAF) from *Laggera aurita* L**

	LM2	S.typ	S.oro	S.inf	S.nig
Gen	500	250	250	500	500
DCMF alone	250	125	125	250	250
EAF alone	250	125	125	125	125
DCMF + Gen	125	62.5	62.5	62.5	125
EAF + Gen	125	31.25	31.25	62.5	62.5

The results are the means of number of the colonies  $\pm$  standard deviations.

**Table 2: Minimum bactericidal Concentration ( $\mu\text{g/ml}$ ) of bioactive fractions (DCMF and EAF) from *Laggera aurita* L**

	LM2	S.typ	S.oro	S.inf	S.nig
DCMF alone	>1000	500	500	1000	>1000
EAF alone	1000	500	500	500	500
DCMF + Gen	500	125	125	250	500
EAF + Gen	250	62.5	62.5	125	125

The results are the means of number of the colonies  $\pm$  standard deviations.

**Table 3: Bacteriocidal/Bacteriostatic capacity of bioactive fractions (DCMF and EAF) from *Laggera aurita* L**

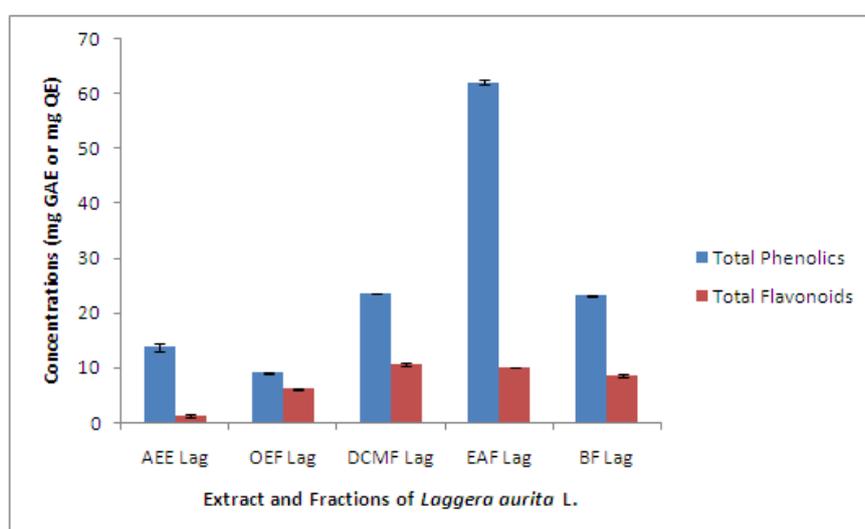
	LM2	S.typ	S.oro	S.inf	S.nig
DCMF alone	-	-	-	-	-
EAF alone	-	-	-	-	-
DCMF + Gen	-	+	+	-	-
EAF + Gen	+	+	+	+	+

The results are the means of number of the colonies  $\pm$  standard deviations., +: bacteriocidal effect (MBC/MIC = 1 or 2) -: bacteriostatic effect (MBC/MIC = 4 or 16).

**Table 4: Fractional Inhibitory Concentration index (FICI) of the best bioactive fraction (flavonoid-rich fractions) in combination with gentamycin against Food Bacterial strains Multi-resistant.**

	LM2	S.typ	S.oro	S. inf	S.nig
FICa	0.50	0.250	0.250	0.50	0.50
FICb	0.250	0.125	0.125	0.125	0.125
FICI	0.750	0.375	0.375	0.625	0.625
Effect	Addi	Syner	Syner	Addi	Addi

FICa = MIC of the combination/MICa alone; FICb = MIC of the combination/MICb alone and FICI = FICa + FICb, a = Flavonoid-rich fractions; b = gentamycin. The FICI was interpreted as follows: (1) a synergistic effect when FICI  $\leq 0.5$ ; (2) an additive effect when FICI  $> 0.5$  and  $< 1$  and (3) an antagonistic effect when FICI  $> 1$ . Syner = Synergetic, Addi = Additive, Gen = gentamycin

**Fig. 1: Polyphenol content of extract and fractions from *Laggera aurita* L.**

**AEE Lag:** Aqueous ethanolic extract of *Laggera aurita*, **OEF Lag:** Oil ether extract of *Laggera aurita*, **DCMF Lag:** Dchloromethane fraction of *Laggera aurita*, **EAF Lag:** ethyl acetate fraction of *Laggera aurita*, **BF Lag:** buthanolic fraction of *Laggera aurita*.

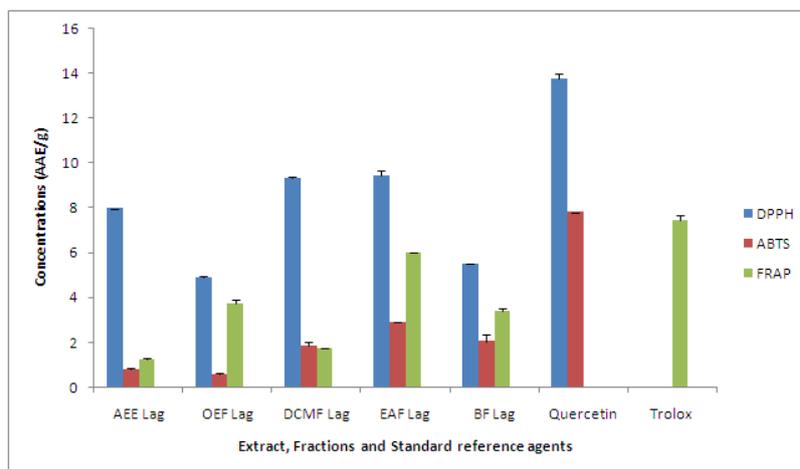


Fig. 2: Antioxidant activity of extract and fractions from *Laggera aurita* L.

**AEE Lag:** Aqueous ethanolic extract of *Laggera aurita*, **OEE Lag:** Oil ether extract of *Laggera aurita*, **DCMF Lag:** Dchloromethane fraction of *Laggera aurita*, **EAF Lag:** ethyl acetate fraction of *Laggera aurita*, **BF Lag:** buthanolic fraction of *Laggera aurita*.

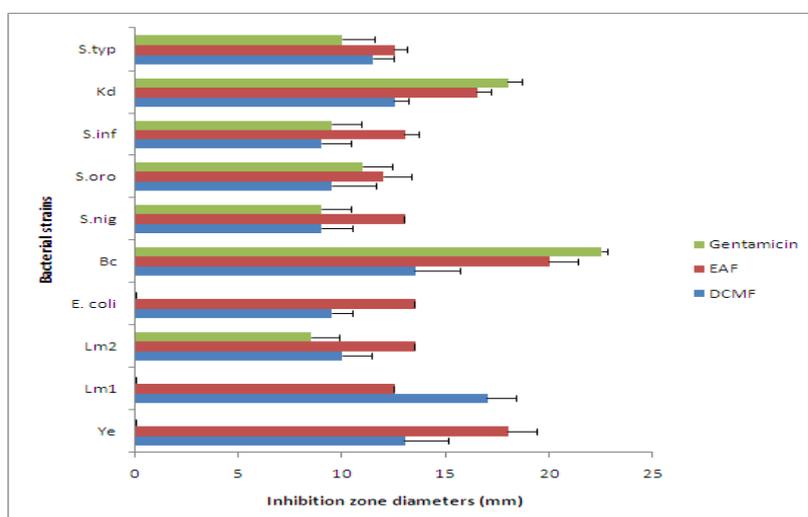


Fig. 3: Inhibition Zone Diameters (mm) of extract and fractions from *Laggera aurita* L. and conventional antibiotics (Penicillin and gentamycin).

## CONCLUSION

This study on this Asteraceae confirms that *Laggera aurita* L. is a good candidate for antibacterial and antioxidant uses. Thus, which many explain the traditional basis of using this herbaceous in the treatment of various bacterial infections in Burkina Faso. Further pharmacological investigations are required to identify the active constituents of the plant extracts responsible for the antioxidant and antibacterial effects.

## CONFLICT OF INTERESTS

Declared None

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## REFERENCES

- Treben, M: Health Through God's Pharmacy. 2<sup>nd</sup> Edn., Ennsthaler Publisher, Australia 1998, pp:260-264.
- Shanmugasundaram, S: Complementary and alternative therapies in palliative care. A transition from modern medicine to traditional medicine in India. J. Cancer Pain Symptom Palliation 2005, 1:25-29.
- Gill, N.S., J. Bajwa, P. Sharma, K. Dhiman and S. Sood: Evaluation of antioxidant and antiulcer activity of traditionally consumed Cucumis melo seeds. J. Pharmacol. Toxicol 2011, 6:82-89.
- Shah, P. M: The need for new therapeutic agents. What is in the pipeline? Clin. Microbiol. Infect 2005, 11:36-42.
- Braca, A., C. Sortino, M. Politi, I. Morelli and J. Mendez: Antioxidant activity of flavonoids from Licania licaniaeflora. J. Ethnopharmacol 2002, 79:379-381.
- Modi, A.J., S.S. Khadabadi, S. L. Deore and M.S. Kbd: Antioxidant effects of leaves of Clerodendrum infortunatum (Linn.). Int. J. Pharmaceut. Sci. Res 2010, 1:67-72.
- Sati, S.C., N. Sati, U. Rawat and O.P. Sati: Medicinal plants as a source of antioxidants. Res. J. Phytochem 2010, 4:213-224.
- Daniel, M: Medicinal Plants. Chemistry and Properties. Science Publishers, USA 2006, pp:1-9.
- Burkill HM: The useful plants of West/Tropical Africa. Royal Botanic Gardens Kew 1985, 1:452-453:481.
- Nacoulma OG: Medicinal plants and their traditional uses in Burkina Faso. PhD Thesis. University of Ouagadougou, Biochemistry-microbiology department 1996.
- Njan Nlôga AM, Saotoing P, Tchouankeu JC, Messi J: Effect of Essential Oils of Six Local Plants Used Insecticide on Adults of *Anopheles gambiae*, Giles 1902, J. Entomol 2007, 4(6):444-450.

12. Okhale SE, Odiniya EO, Kunle OF: Preliminary Phytochemical and Pharmacognostical Investigation of Pediatrics Antimalarial *Laggera pterodonta* (DC) Sch. Bip.: Asteraceae of Nigerian Origin, Ethnobot. Leaf 2010, 14:457-66, 2010.
13. Zhao Y, Reanmongkol W, Bouking P, Zhang R: Acute toxicity and antinoceptive effect of *Laggera pterodonta* (DC) Benth Aqueous extract. Nat. Prod. Res. Dev 2004, 17(4):457-459.
14. Shi S, Huang K, Zhang Y, Zhao Y, Du Q: Purification and identification of antiviral components from *Laggera pterodonta* by high-speed counter-current chromatography. J. chromatogr. B. Analytical technologies in the biomed. Life. Sci 2007, 859 (1):119-124.
15. Wu Y, Wang F, Zheng Q, Lu L, Yao H, Zhou C, Wu X, Zhao Y: Hepatoprotective effect of total flavonoids from *Laggera alata* against carbon tetrachloride-induced injury in primary cultured neonatal rat hepatocytes and in rats with hepatic damage. J. Biomed. Sci 2006, 13:569-578.
16. Yang GZ, Li YF, Yu X, Mei ZN: Terpenoids and flavonoids from *Laggera pterodonta*, Yao. Xue. Xue. Bao 2006, 42(5):511-515.
17. Xiao Y, Zheng Q, Zhang Q, Sun H, Guéritte F, Zhao Y: Eudesmane derivatives from *Laggera pterodonta*. Fitoterapia 2003, 74(5):459-463.
18. Donatien Kaboré, Hagrétou Sawadogo-Lingani, Mamoudou H. Dicko, Bréhima Diawara and Mogens Jakobsen: Acid resistance, bile tolerance and antimicrobial properties of dominant lactic acid bacteria isolated from traditional "maari" baobab seeds fermented condiment. African Journal of Biotechnology 2012, 11(5):1197-1206
19. Lamien-Meda A., C.E. Lamien, M.M.Y. Compaoré, R.T.N. Meda, M. Kiendrebeogo, B. Zeba, J.F. Millogo and O.G. Nacoulma: Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 2008, 13:581-594.
20. Hinnebourg, I., Damien Dorman, H. J., Hiltunen, R.: Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry 2006, 97:122-129.
21. Konaté K, Hilou A, Mavoungou JF, Lepengué AL, Souza A, Barro N, Datté JY, M'Batchi B and Nacoulma OG: Antimicrobial activity of polyphenol-rich fractions from *Sida alba* L. (Malvaceae) Against cotrimoxazol-resistant bacteria strains. Annals of Clinical Microbiology and Antimicrobials 2012, 11:5. doi:10.1186/1476-0711-11-5.
24. NCCLS-National Committee for Clinical Laboratory Standards. Performance standard for anti-microbial susceptibility testing: eleventh informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standard, Wayne, PA, USA 2001.
25. Berche P, Gaillard JL, Simonet M: In Nosocomial Infections Caused by Bacteria and Their Prevention in Bacteriology Edited by: Flammarion Medicine Sciences 1988, 64-71.
26. Kiessoun Konaté, Jacques François Mavoungou, Alexis Nicaise Lepengué, Raïssa RR Aworet-Samseny, Adama Hilou, Alain Souza, Mamoudou H Dicko and Bertrand M'Batchi: Antibacterial activity against  $\beta$ -lactamase producing Methicillin and Ampicillin-resistant *Staphylococcus aureus*: fractional Inhibitory Concentration Index (FICI) determination BMC, Annals of Clinical Microbiology and Antimicrobials 2012, 11:18 doi:10.1186/1476-0711-11-18.
27. Rice-Evans, C.A., N. Miller and G. Paganga: Antioxidant properties of phenolic compounds. Trends Plant Sci 1997, 2:152-159.
28. Prior, R.L. and G. Cao: Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. Hort. Sci 2000, 35:588-592.
29. Mathew, S. and T. E. Abraham. In vitro antioxidant activity and scavenging effect of Cinnamomum verum leaf assayed by different methodologies. Food. Chem. Toxicol 2006, 44:198-206.
30. Meenakshi, S., D.M. Gnanambigai, S.T. Mozhi, M. Arumugam and T. Balasubramanian: Total flavonoid and in vitro antioxidant activity of two seaweeds of rameshwaram coast. Global J. Pharmacol 2009, 3:59-62.
31. Machado, T.D., I.C.R. Leal, A.C.F.A. Maral, K.R.N. dos Santos, M.G. da Siva and R.M. Kuster: Antimicrobial ellagitannin of *Punica granatum* fruits. J. Brazilian Chem. Soc 2002, 13:606-610.
32. Cakir, A., A. Mavi, A. Yildirim, M.E. Duru, M. Harmandar and C. Kazaz: Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. J. Ethnopharmacol 2003, 87:73-83.
33. Duh, P.D., Y.Y. Tu and G.C. Yen: Antioxidative activity of water extracts of Hamg jzur (*Chrysanthemum morifolium*). Lebensmittel-Wissenschaft Technol 1999, 32:269-277.
34. Tanaka, M., C., W. Kuie, Y. Nagashima and T. Taguchi: Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. Bull. Jap. Soc. Scientific Fisheries 1988, 54:1409-1414.
35. Aneta, W., O. Jan and C. Renata: Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 2007, 105:940-949.
36. Schlesier, K., M. Harwat, V. Bohm and R. Bitch: Assessment of antioxidant activity by using different in Vitro methods. Free radical Res 2002, 36:177-187.
37. Nsimba, R.Y., H. Kikuzaki and Y. Konishi: Antioxidant activity of various extracts and fractions of chenopodium quinoa and Amaranthus Ssp. Seeds. Food chem 2008, 106:760-766.
38. Anna, M.N., P.P. Riitta, A. Marjukka and O.C. Kirsijärvi: Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food chem 2003, 81:485-493.
39. Negro, C.L. Tommasi and A. Miceli: Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol 2003, 87:41-44.
40. Ramandeep, K.T. and P.S. Geoffrey: Antioxidant activity in different fraction of tomatoes. Food Res. Int 2005, 38:487-494.
41. Gao, X., M. Ohlander, N. Jeppsson, L. Björk and V. Trajkovski: Changes in antioxidant effects and their relationships to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J. Agric. Food Chem 2000, 48:1485-1490.
42. Hoffman, D.L.: The Herb User's Guide. Thomson Publishing Group, Wellingborough, London 1987.
43. Nasar-Abbas, S.M. and A.K. Halkman: Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. Int. J. Food Microbio 2004, 97:63-69.
44. Rahman, A., M.A.K. Parvez, T. Parvin, D.K. Paul and M.A. Sattar: Antimicrobial activity of three bioactive compounds from the stem bark of Piper chaba hunter. Bioscience Res 2004, 1:16-20.
45. Abeer M, Haj A, Sanaa OY: Anti-microbial Activity of Acacia nilotica Extracts Against Some Bacteria Isolated from Clinical Specimens. Res J Med Plant 2007, 1(1):25-28.
46. Rabe T, Mullholland D, van Staden J: Isolation and identification of antibacterial compounds from *Vernonia colorata* leaves. J. Ethnopharmacol 2002, 80:91-94.
47. Machado TD, Leal ICR, Amaral ACF, dos Santos KRN, da Siva MG, Kuster RM: Antimicrobial ellagitannin of *Punica granatum* fruits. J Braz Chem Soc 2002, 13:606-610.
48. Cowan MM. Plants products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-582.
49. Kelmanson, J. E., A. K. Jager and J. van Staden: Zulu medicinal plants with antimicrobial activity. J. Ethnopharmacol 2000, 69:241-246.
50. Zongo, C., A. Savadogo, L. Ouattara, I.H.N. Bassole and C.A.T. Ouattara: Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): A medicinal plant from Burkina Faso. Int. J. Pharmacol 2010, 6:880-887.
51. Reynolds, J.E.F.: Martindale the Extract Pharmacopoeia. 31<sup>st</sup> Edn., Royal Pharmaceutical Society of Great Britain, London 1996.
52. Bezic, N.M., S.V. Dinkic and A. Radonic: Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. Phytother. Res 2003, 17:1037-1040.
53. Tomas-Barberan, F., E. Iniesta-Sanmartin, F. Tomas-Lorente and A. Rumbero: Antimicrobial phenolic compounds from three Spanish *Helichrysum* species. Phytochemistry; 29:1093-1095.
54. Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA: Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Applied Microbiol 2000, 30:379-384.
55. Bachi BB, Rotherer S: Factors influencing methicillin resistance in *Staphylococci*. Arch Microbiol 2002, 178:165-171.
56. Hooper DC: Emerging mechanisms of fluoroquinolone resistance. Emerg Infect Dis 2001, 7:337-341.