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

Crépin I Dibala
KIESSOUN KONATÉ
MAMOUNATA DIAO
MAURICE OUÉDRAOGO
Mamoudou H. DICKO, Prof.
INTRODUCTION

The use of plants in the management and treatment of diseases shared 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].

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

CRÉPIN LDIBALA*,1, KIESSOUN KONATÉ2, MAMOUNATA DIAO1, MAURICE OUÉDRAOGO1 AND MAMOUDOU H.DICKO1

1Laboratory of Food Biochemistry, Enzymology, Biotechnology and Bioinformatic, University of Ouagadougou, 03 P.O.Box: 848 Ouagadougou 03, Burkina Faso. 2Unit of Formation in Sciences Applied and Technological (UFR/SAT) and Institute of Sciences of the Environment and the Rural Development (ISEDR), Polytechnic University of Dédougou, Burkina Faso. 3Laboratory of Animal Physiology, University of Ouagadougou, 09 PO Box: 848 Ouagadougou 09, Burkina Faso.

Email: mehekiessoum@yahoo.fr

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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 cicoalteau and A1C3 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, Z- 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-resistants 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, EAE 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 propertries.

MATERIALS AND METHODS

Materials

Chemicals and reagents

The Folin-cicloalteau reagent, NaH2PO4, Na2HPO4, sodium carbonate, aluminium trichloride, gallic acid and quercetin were purchased from Sigma-aldrich chemie, Steinheim, Germany.
2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, and solvents used were from Fluka Chemie, Switzerland. Potassium hexacyanoferrate \([K_3Fe(CN)_6]\) was from Prolabo and ascorbic acid was from Labosip, 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, \(Lm = Listeria monocytogenes\) 057, \(Lm = Listeria monocytogenes\) Scott A, S. Inf = Salmonelle infantis SKN 557, \(Soro = Salmonella oraniensis\) SKN 1157, \(Sntg= Salmonella Nigeria\) SKN 1160, \(Stypo = Salmonella typhimurium\) SKN 533, \(Es= Escherichia coli\) S81 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, Switzerland) 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, Switzerland) at approximately 40°C. The aqueous extracts were subjected to sequential liquid-liquid extraction with oil ether, dichlormethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain oil ether fraction (OEF), dichlormethane fraction, ethyl acetate fraction 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**

The total phenol was estimated according to the Dowd method as adapted by [19]. 0.5 ml of methanolic AlCl\(_3\) (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 760 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\(^2\) = 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) per 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\(^2\) = 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 7 mM concentration. ABTS radical cation (ABTS\(^\cdot+\)) 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 mg/ml in methanol) which was allowed to react with 990 µL of fresh ABTS\(^\cdot+\) solution and the absorbance was taken 6 min exactly after initial mixing. Ascorbic acid was used as standard (Y = -0.034x + 0.634; R\(^2\) = 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 mg/mL-1) was mixed with 1.25 ml of phosphate buffer (0.2M, pH 6.6) and 1.25 ml of aqueous potassium hexacyanoferrate \([K_3Fe(CN)_6]\) solution (1%). After 30 min incubation at 50°C, 2.5 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\(_3\) 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\(^2\) = 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. Trolox, 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 McFarland standard (10⁸ CFU/ml). To obtain the inocula, these suspensions were diluted 100 times in Muller Hinton broth to give 10⁶ colony forming units (CFU)/ml.

Preparation of discs
The stock solutions of extract or each fraction were dissolved in 10 % dimethyl sulfoxide (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 antibacterial 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 (10⁶ CFU/ml). After drying in a sterile hood, 6 mm diameter discs soaked with 10 μl of the different extract solutions 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°8) served as sterility controls (contained broth only) or negative control (broth + inoculum). 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. (Asteraceae) 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 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 + 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 calculation 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 lagerra aurita L. extract fractions ranged from 62.12±0.68mgGAE to 9.07±0.17mgGAE. The highest content of total phenolics was detected in EAF with 62.12±0.68mgGAE following by DCFM with 23.67±0.05mgGAE. The lowest total phenolics were obtained in OF with respectively 9.07±0.17mgGAE.

The total flavonoids content per 100 mg of lagerra aurita L. extract and fractions ranged from 10.56±0.29mgQE to 1.28±0.20mgQE. The highest content of total flavonoids in lagerra aurita L. was detected in EAF with 23.67±0.05mgGAE and OE F with 21.34±0.14mgGAE. The lowest total flavonoids were detected in OF with respectively 1.28±0.14mgGAE. 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 AAEE/g extract to 4.907± 0.0587 mmol AAEE/g extract). From these result, the strongest DPPH activity was obtained by EAF with 9.472 ± 0.1941 mmol.
mmol AAE/g extract followed by DCMF with 9.34±7.06 mmol AAE/g extract. The lowest activity was obtained by EAF with 4.907±0.030 mmol AAE/g extract. Control compound gave 13.76±0.26 mmol AAE/g extract for Quercetin.

For ABTS assay, the following were values obtained respectively 0.78±0.083 mmol AAE/g extract for EHA, 0.58±0.04 mmol AAE/g extract for EAF, 2.93±0.308 mmol AAE/g extract for DCMF, 2.93±0.030 mgGAE/100mg extracts for EA, and 1.85±1.43 GAE/100 mg extract for BF. From these result, the strongest ABTS activity was obtained by EAF with 2.93±0.030 mmol AAE/g extract followed by DCMF with 2.03±0.308 mmol AAE/g extract. The lowest activity was obtained by EAF with 0.58± 0.04mg GAE/100 mg extract. The reference compound is Quercetin 7.81 ± 0.21 mmol AAE/g extract.

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 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 antioxidants [30]. Also, Phenols are very important scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots ad bark [27]. There are many synthetic antioxidants in the market that are used in a wide range of microorganisms. Phenol is well known as a chemical antibacterial agent, 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 antibacterial agent [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 hydrophobic 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 sensitive on certain bacteria strains than standard drug (gentamicin). 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 β-lactam antibiotics due to the production of chromosomal or plasmid mediated β-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 β-lactams because PBP2 has a decreased binding affinity to β-lactams [53]. This has resulted in the development of multiresistance against β-lactams and other antibiotics. In addition, the polysaccharide capsular material in some of the pathogenic microorganisms is responsible for virulence and antimicrobial resistance [54].
Table 1: Minimum Inhibitory Concentration (µg/ml) of bioactive fractions (DCMF and EAF) from Laggera aurita L

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The results are the means of number of the colonies ± standard deviations.

Table 2: Minimum bactericidal Concentration (µg/ml) of bioactive fractions (DCMF and EAF) from Laggera aurita L

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The results are the means of number of the colonies ± standard deviations.

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

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The results are the means of number of the colonies ± standard deviations. +: bactericidal 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-resistants.

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</table>

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 ≤0.5; (2) an additive effect when FICI >0.5 and <1 and (3) an antagonistic effect when FICI >1. Syner = Synertistic, Addi= Additive, Gen = gentamycin

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

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


