Original Research Article

Phytochemical screening and antibacterial activity of solid residues from hydrodistillation of *Cymbopogon nardus* from Burkina Faso

ABSTRACT

The hydrodistillation of aromatic plants is an extraction process that provides essential oils and also generates unexploited solid and liquid residues. The present study focussed on knowledge on the phytochemical profile and antibacterial potential of the solid residue resulting from the hydrodistillation of *Cymbopogon nardus* (CN) from Burkina Faso, with the aim of its valorization. Ethanolic extract was prepared for phytochemical screening using Thin Layer Chromatography, and the total phenolic and flavonoid contents were determined using the Folin-Ciocalteu and aluminum chloride ($AlCl_3$) methods, respectively. The antibacterialpotential was evaluated *in vitro* on six (06) bacterial strains. A variety of phytochemical compounds, including flavonoids, tannins, triterpenes, and coumarins, were identified in the ethanolic extract of the residue from the hydrodistillation of Cymbopogon. The total polyphenol and flavonoid contents were 42.69±6.88 mg GAE/g and 47.82±3.42 mg QE/g of dry ethanolic extract, respectively. In terms of antibacterial potential, the CN extract showed antibacterial activity (diameter ≥8) against *S. aureus*, *S. typhimurium*, *E. coli*, and *S. saprophyticus* strains. These results indicate that the hydrodistillation residue of Cymbopogon nardus contains bioactive molecules and could be used as an agent to combat bacterial contamination in food.

Keywords: hydrodistillation co-product ; phytochemical screening ; biopesticide ; Cymbopogon nardus ; antibacterial activity.

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1. INTRODUCTION

Agricultural production, an important sector of socio-economic development in many countries, continues to face numerous biotic and abiotic constraints [1]. These constraints include microorganisms such as bacteria, which can affect crops and post-harvest production, causing numerous public health problems [2-4]. The presence of these bacteria in agricultural products remains a food safety concern. In 2006, an epidemic caused by the consumption of spinach (*Spinacia oleracea*) infected by *Escherichia coli* resulted in 205 infections and 3 deaths in the United States and Canada [5]. Bad post-harvest processing and storage can lead to the appearance of bacteria such as *Staphyloccocus aureus*. In Burkina Faso, several cases of epidemics caused by food-borne pathogens such as salmonella or *E. coli* are still reported [6]. Several bacterial

pathogens, such as *Pseudomonas syringae*, *Clavibactermichiganensis*, *Xanthomonas axonopodis*, can affect tomatoes and cause yield losses ranging from 52 to 90% [7].
Specialized plant metabolites such as flavonoids, phenolics, terpenoids, saponins and nitrogen compounds are known for their anticancer, antibacterial, anti-inflammatory, antifungal and other biological properties [8]. With the aim of proposing environmentally-friendly treatments to protect crops and post-harvest production against these infectious agents, plant extracts could be exploited for healthy, sustainable agricultural practice.
Hydrodistillation, an extraction technique applied to aromatic plants, produces essential oils. The process also generates solid and liquid residues. However, these co-products could be put to good use, as they are rich in bioactive compounds such as polyphenols and flavonoids. Exploiting plant residues in protective formulations for agricultural products during production, preservation or processing would reduce production costs in an environmentally-friendly way. Studies on the hydrodistillation residues of aromatic plants and their valorization exist, but they are few [9-12]. Flavonoids are known for their anti-free radical, antifungal, anti-inflammatory, antibacterial, antimicrobial, anticarcinogenic and antipyretic properties [13]. In a previous study, the antitermite activity of hydrodistillation residues from *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Mentha piperita* was demonstrated [14]. The biological efficacy of ethanolic extracts of these solid residues has been demonstrated, and chemical analysis has shown them to be rich in phenolic compounds, particularly flavonoids.
The present study focuses on *Cymbopogon nardus*with a view to valorizing its solid residue from hydrodistillation. To this end, *(i)* certain phytochemical constituents were identified by thin layer chromatography (TLC), *(ii)* total phenolic and total flavonoid contents were determined and finally *(iii)* antioxidant potential and antibacterial activity were assessed in comparison with *C citratus*.

2. MATERIAL AND METHODS

2.1.Solvents and reagents

Solvents used were bioethanol 70%, acetic acid (PROLABO), distilled water; ethyl acetate, dimethyl sulfoxide (DMSO), hydrochloric acid 37% from Sigma Aldrich; methanol (HPLC grade), formic acid (CARLO ERBA).
The standards and reagents used are gallic acid, PEG (polyethylene glycol), aluminum chloride hexahydrate, DPPH (2, 2 - diphenyl - 1 - picrylhydrazil), Folin - Ciocalteu reagent, TPTZ (2, 4, 6 - tri [2 - pyridyl] - s - triazine), potassium persulfate, trolox (6 - hydroxy - 2,5,7,8 - tetramethylchroman - 2 - carboxylic acid)from Sigma Aldrich ; quercetin from Merck; Neu reagent (2 - aminoethyldiphenylborate) from ROTH; sodium carbonate from Emsure; ABTS (2,2' - azino - bis (3 - ethylbenzothiazoline - 6 sulphonic acid) from BioChemica; sodium acetate from Techno Pharmchem and iron III chloride from CARLO ERBA.

2.2. Plant material

The aerial parts of *Cymbopogon nardus* (L.) (Rendle, 1753) were collected from a cultivation plot at the Permaculture Lawatancenter of Bobo Dioulasso. After collection, the leaves were washed and then dried under ventilated conditions in the shade for two (02) weeks. The dry leaves obtained were then ground to a fine powder using an electric grinder. These powders are stored in airtight boxes for further use.

2.3. Ethanolic extract preparation

Hydrodistillation was carried out using 150 g of powder and 1.5 L of distilled water, placed in a 3 L flask and boiled for 1 h 30 min. After cooling, the mixture was filtered through a funnel fitted with filter paper, and the solid residue was dried under ventilation for 72 h.One hundred grams (100 g) of this residue was mixed with 1 L of 70% ethanol and left under stirring for 24 h. After filtration, the resulting solution was concentrated at reduced pressure using a rotary evaporator and kept cool for 48 h to precipitate the vegetable fats.

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Finally, after further filtration followed by evaporation to dryness using a rotary evaporator, the solid extract labelled ECN (ethanolic extract of *Cymbopogon nardus*) was used for the various analyses.

2.4. Phytochemical screening by thin layer chromatography (TLC)

The various phytochemical groups were identified using specific solvent systems and reagents for each group :
- *Alkaloids* : solvent s*ystem*, AcOEt -MeOH- H_2O (18:1:1, v/v/v) ; Reagent of revelation, Dragendorff[15].
- *Coumarins* : solvent system*,* AcOEt-HCOOH-AcOH-H_2O (100 : 11 : 11 : 20, v/v/v/v) ; Reagent of revelation*,* KOH éthanolique 5 – 10% [16].
- *Flavonoids* :solvent system, AcOEt-HCOOH-AcOH-H_2O (100 : 11 : 11 : 20, v/v/v/v) ; Reagent of revelation, $AlCl_3$ (0,5 g/100 mL de EtOH) et Neu/PEG [15].
- *Tannins* : solvent system: n- BuOH-AcOH/H_2O (4:1:5, v/v/v) ; Reagent of revelation*,* $FeCl_3$ (10% in MeOH-H_2O, 1:1, v/v) [17].
- *Sugars* : solvent system, AcOEt-HCOOH-AcOH-H_2O (100 : 11 : 11 : 20, v/v/v/v) ; Reagent of revelation, thymol sulfurique (0,5 g of thymol with 95 mL of ethanol then add 5 mL of sulfuric acid concentred) [16].
- *Triterpenes* : solvent system*,* AcOEt-HCOOH-AcOH-H_2O (100 : 11 : 11 : 20, v/v/v/v) ; Reagent of revelation*,* Lieberman – Burchard (5 mL of anhydride acetic, 5 mL of concentredsulfuric acid and 50 mL of EtOH 95%) [16].
The colors of triterpene stains are orange and yellow-orange at UV-365 nm [15]. Tannins fluoresce blue at UV-365 nm [17]. Pink stain colors reveal the presence of sugars [16]. The presence of coumarins has been demonstrated by the appearance of blue and brown fluorescence [16]. Orange and yellow fluorescence observed at UV-365 nm and revealed with the Neu/PEG reagent, show the presence of glycosylated flavonoids in the extract

2.5. Determination of total phenolic content

The total polyphenol content of the extracts was determined using the colorimetric method of Folin - Ciocalteu [18]. A cascade dilution of aqueous extract solutions was carried out starting at 1mg/mL. To 50 µL of each solution obtained, 50 µL mL of Folin - Ciocalteu reagent (2 mL diluted 10-fold in distilled water) was added, and 8 minutes later, 100 µL of sodium carbonate (1.6 mL 7.5% Na_2CO_3 in distilled water) was added. After 1 h incubation at room temperature, absorbance was measured at 765 nm. A calibration curve was established using gallic acid as the standard. All preparations and analyses were performed in triplicate. Values are expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

2.6. Determination of total flavonoids content

The flavonoid content of the extracts was determined by the $AlCl_3$ aluminum chloride assay [19]. One hundred 100 µL of $AlCl_3$ 2% aluminum chloride in methanolic solution was added to 100 µL of plant extract. A cascade dilution was performed starting from 1mg/mL. After 40 min incubation at room temperature, absorbance was measured at 420 nm. A methanolic solution was used as a blank. A calibration curve was established using quercetin as standard. All preparations and analyses were performed in triplicate. Values are expressed in milligram quercetin equivalent per gram dry extract (mg QE/g).

2.7. LC-MS analysis

All LC-MS analyses were carried out using an HPLC chain (Waters Allinace 2695) coupled to the Waters QToF-Premier with an Electrospray ionization(ESI) source in negative mode. A Phenomenex Kinetex C18 EVO reversed-phase HPLC column (150 x 2.1 mm i.d., 100 Å particle size) was used.Solvent system: acetonitrile (solvent A) and water + 0.01% formic acid (solvent B) with a gradient described as follows: A = 10 %, B = 90 % at t = 0 min; A = 30 %, B = 70 % at t = 5 min; A = 35 %, B = 65 % at t = 10 min; A =

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50 %, B = 50 % at t = 15 min; A = 90 %, B = 10 % at t = 20 min; A = 100 %, B = 0 % at t = 22 min; A = 100 %, B = 0 % at t = 24 min, A = 10 %, B = 90 % at t = 25 min.Capillary and cone potentials were -3.1 kV and -30 V respectively. The mass ion range *m/z* 50-2000 was analyzed using the ToF (Time-Of-Flight) analyzer. The chromatograms obtained were processed using MassLynx V 4.1 software (Waters, Manchester, UK)

2.8. Antioxidant activity evaluation

2.8.1. DPPH radical scavenging

The scavenging activity of the extracts was assessed using the DPPH free radical method (DPPH$^{\cdot}$) described in the literature [20] and adapted to microplates. DPPH radicals have a maximum absorption at 515 nm, which disappears with reduction by an antioxidant compound. The DPPH radical solution in methanol (6 x 10-5 M) was prepared just prior to use by dissolving 2.36 mg DPPH in 100 mL methanol. A range of extract solutions was prepared, with concentrations ranging from 1mg/mL to 0.0079 mg/mL. To 100 µL of methanolic solution of each plant extract was added 100 µL mL of DPPH radical solution. Samples were incubated for 20 min at room temperature, then the decrease in absorbance at 515 nm was measured (AE). Results are expressed as inhibitory concentration IC_{50} (mg/mL). All measurements were made in triplicate. Trolox was used as a reference. The radical inhibition rate was calculated according to the following formula: % DPPH inhibition= [(AB AE)/ AB] x 100

2.8.2. Radical ABTS decoloration method

The ABTS method described by Re et al (1999) was also used to assess the antioxidant activity of the extracts [21]. The ABTS mono cation radical was performed by oxidation of the ABTS salt solution (7 mM) with potassium persulfate solution (2.45 mM) (ratio 1 : 0.5). Results are expressed as inhibitory concentration IC_{50} (mg/mL).The mixture was left to react for 16 h at 30°C. Then 2 mL of the solution were diluted in 28 mL ethanol to give an absorbance of 0.7 ± 0.02 at 734 nm. One hundred and ninety microliters (190 µL) were added to 10µL of extracts. All measurements were made in triplicate. Results are expressed as inhibitory concentration IC_{50} (mg/mL). Trolox was used as a reference. Percent inhibition was calculated using the equation described in the DPPH method.

2.8.3. FRAP method

The antioxidant capacity of the extracts was determined spectrophotometrically using the method of Benzie and Strain (1996) [22]. For absorbance measurement, 300 µL of freshly prepared FRAP reagent was mixed with 10 µL of extract and 30 µl of water. Absorbance was measured at 593 nm 5 min later.A calibration curve was established using trolox as the standard. All preparations and analyses were carried out in triplicate. Values are expressed in milligrams of trolox equivalent per gram of dry extract (mg TE/g).

2.9. Antibacterial activity evaluation

The antimicrobial potential of the extracts was assessed by diffusion of impregnated discs solubilized in DMSO to a solution of 100 mg/mL. Tests were carried out on Muller Hilton (MH) agar for strains(*Escherichia coli*ATCC 8739, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus*ATCC 6538, *Staphylococcus saprophyticus*ATCC 2593, *Salmonella typhimurium* ATCC14028).Twenty (20) mL of sterilized MH agar and Sabouraud agar culture media, cooled to 45-50°C, were dispensed into sterile 9 cm Petri dishes. After solidification, a standardized microbial suspension (0.5 McFarland) was inoculated with a sterile swab. Sterile Wattman paper discs 1, 6 mm in diameter, were impregnated with 10 µL of extract and deposited with sterile forceps on the agar surface, which had been previously seeded.Petri dishes were kept at 4°C for 2 h and then incubated at 37°C for 24 h for bacteria. The antimicrobial activity of the extracts was determined by the presence and measurement of an inhibition halo around the extract-impregnated disc. Results were expressed in millimeters (mm).

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Chloramphenicol (30 µg) was used as a positive control for bacterial strains. Tests were performed in duplicate [23].

2.9.1. Determination of Minimum Inhibitory Concentration (MIC)

Only microbial strains sensitive to active extracts were selected to determine the minimum inhibitory and bactericidal concentration by microdilution in liquid medium.
In 96-well microplates, 100 µL of sterile Müller-Hinton broth medium was added to 100 µL of the 100 mg/mL extract, followed by cascade dilution.One hundred (100) µL of bacterial/fungal inoculum was then added to the mixture. The negative control consisted solely of the extract and Muller-Hinton broth. The Muller-Hinton broth/bacterial suspension mixture served as the positive control. After incubation at 37°C for 24 h, the reading was taken by colorimetric assay using 40 µL of p-iodonitrotetrazolium (INT at a concentration of 0.2 mg/mL after incubation for 30 min) [23].

2.9.1. Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC), is the lowest concentration of an antimicrobial agent that leaves no more than 0.01% of germs alive. One hundred (100) µL of the mixture was taken from wells with a concentration greater than or equal to the MIC and plated onto Mueller Hinton agar, then incubated for 24 h at 37°C. The lowest concentration at which no growth was observed on the agar after 24 h of incubation was considered to be the MBC. MBC/MIC ratios were calculated to assess the bactericidal or bacteriostatic activity of active extracts on the basis of the following criteria [23] :

- MBC/MIC ≤ 4 : the extract has bactericidal power
- MBC/MIC >4 : the extract has bactériostatic power

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening by TLC

Phytochemical screening was carried out on the ethanolic extract of the solid residue from the hydrodistillation of *Cymbopogon nardus* (ECN) leaves; Figure (1) shows the positive results observed on TLC plates made with the different solvent systems according to the chemical family targeted.The presence of spots in TLC revealed the presence of several families of phytochemical compounds : flavonoids, triterpenes, sugars and coumarins. Alkaloids were absent.
Several spots are visible on the TLC plates, indicating the richness of compounds from various families present in the solid residue. In the case of flavonoids, the position of the spots indicates the presence of glycosylated flavonoids. Indeed, luteolin - 6 - C - arabinoside - 8 - C - glucoside, luteolin -6,8 - di - C - arabinoside, orientin and vitexin have been reported to be present in *Cymbopogon nardus* [24].Romero et al. also found luteolin-6-C-glucoside (6.0± 0.5 mg /g extract), luteolin-O-desoxyhexosyl-C-hexoside (5.9± 0.1 mg /g extract), and luteolin-C-hexoside-C-pentoside (5.1 ±0.2 mg /g extract) [25].

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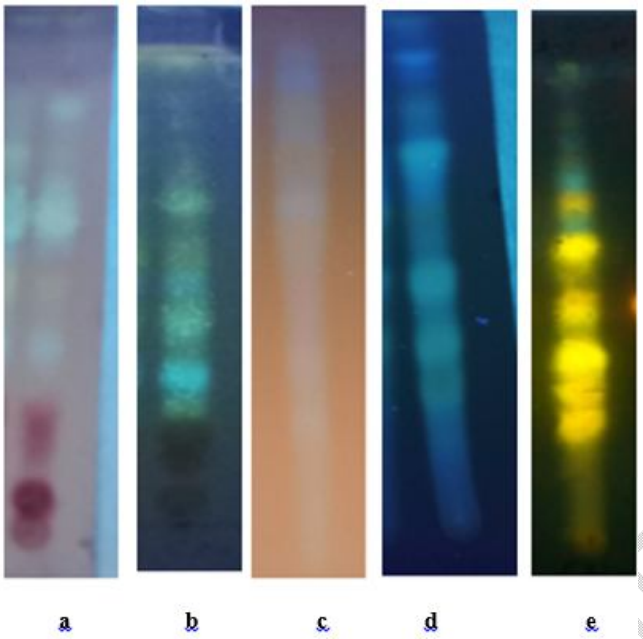

Fig.1. TLC detection of phytochemical groups in the ethanolic extract of *Cymbopogon nardus*. a : Sugars ; b : Triterpenes ; c : Tannins ; d : coumarins ; e : Flavonoids

3.2. Total phenolics and total flavonoids content

The total phenolic and flavonoid contents of the solid hydrodistillation residue of *C. nardus* are given in Table 1 in comparison with the *Cympopogoncitratus* data studied previously [14].This result shows that the solid residue of *C. nardus* presented a significant content of total phenolic compounds, i.e. around 4% of the dry extract. This remains below the levels observed for *C. citratus*.
These results confirm that ethanol is a good extraction solvent of flavonoids [26], because of the significant content of total flavonoids of 47.82±3.42mg QE/g of dry extract of*C. nardus*solid residue.

Table 1. Total phenolic compound and flavonoid content of ethanolic extract of *Cymbopogon nardus* and *Cympopogoncitratus*

Species	Total phenolics content (mg GAE/g)	Total flavonoids content (mg QE/g)
C. nardus	42.69±6.88	47.82±3.42
C. citratus (previouswork)	74.17 ±13.53	185.19 ± 16.9

3.3. LC-MS profil of *C. nardus*

Phenolic compounds were sought in *C. nardus* leaf extract by high performance liquid chromatography – mass spectrometry analysis. Several peaks were observed at mass to charge *m/z* ratios between 265 and 693. The chromatogram in figure (2) corresponds to the total ionic current (TIC) recorded for the phenolic acid and flavonoid retention time range from 0 to 16 min. The extracted chromatograms for each compound, together with their mass spectra (figure 1).The low *m/z* values could probably correspond to phenolic aglycones, or either to fragment ions which could arise from the in-source dissociations of high molecular weight compounds. The peak at tr 14. 9 min gives an *m/z* value that did not lead to compound identification. Only two peaks with high mass-to-charge ratios, namely *m/z* 693 at tr = 10.9 min and *m/z* 567 at tr = 16.8 min, were explored. They do not

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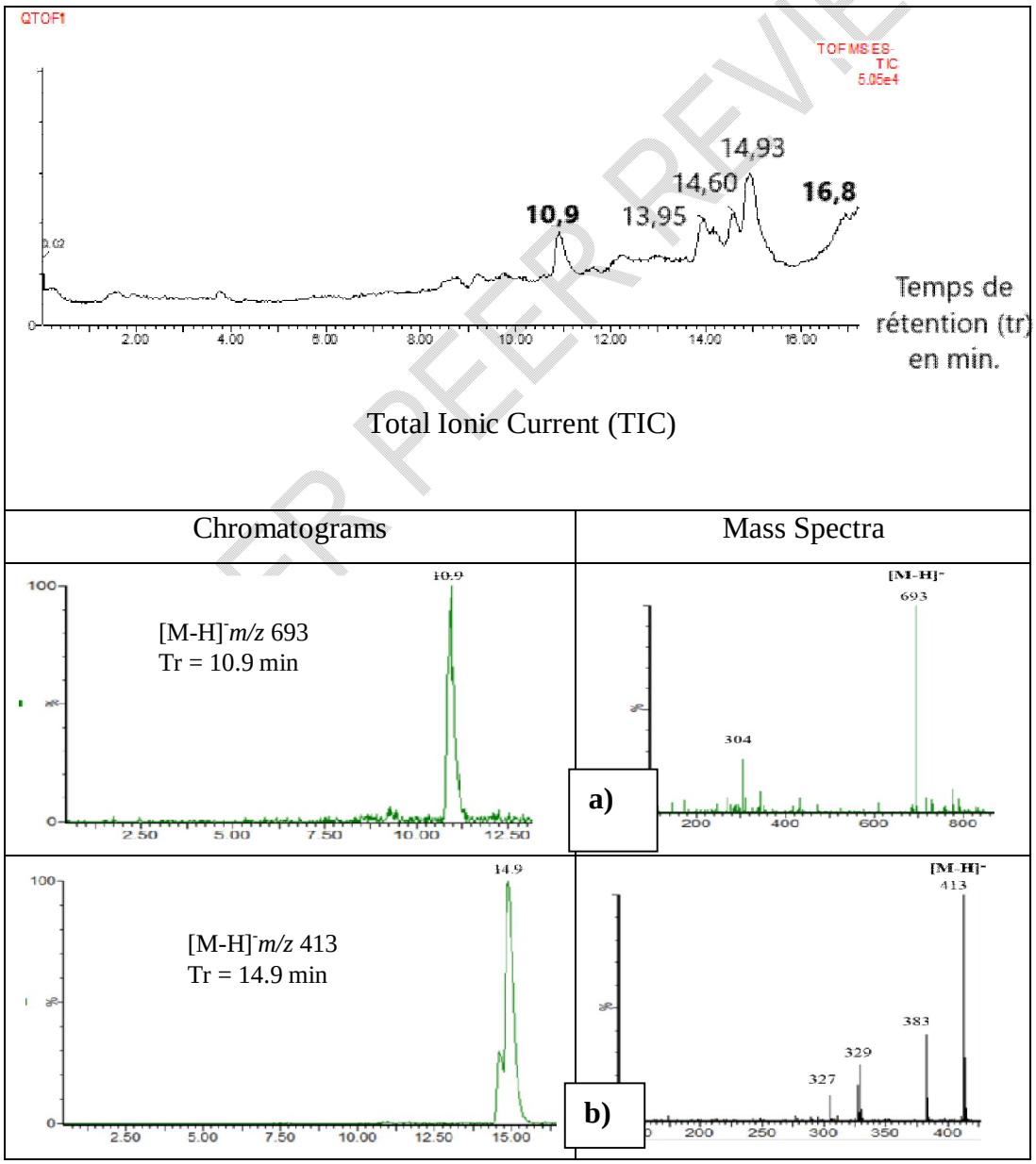
correspond to the *m/z* values of compounds already identified in *C. nardus*, namely luteolin-6-C-rabinoside-8-C-glucoside, luteolin-6,8-di-C-rabinoside, luteolin-8-C-glucoside (orientin) and apigenin-8-C-glucoside (vitexin) [24], luteolin-6-C-glucoside (iso-orientin), luteolin-O-desoxyhexosyl-C-hexoside and luteolin-C-hexoside-C-pentoside with expected *m/z* of 609, 549, 447, 431, 447, 593 and 609 respectively [25].

However, literature data were used to attempt to identify these two compounds. Indeed, the peak at *m/z* 693, corresponding to the presence of a major peak at [M-H]- in the analysis of *Dipcadierythraeum* extracts, made it possible to identify the apigenin 6-C-pentoside 8-C-rhamnose O-rhamnoside after LC-MS/MS analysis [27].The [M-H]- compound with *m/z* 567 was identified in *Helichrysum obconicum* extracts. Further investigations identified a dimethoxyl-flavanone derivative [28].

Analysis, including Collision Induced-Dissociation (CID) by tandem analysis using different collision energies, is required to confirm the exact identity of these flavonoid derivatives. Nevertheless, these clues give an idea of the flavonoid profile of the *C. nardus* species, in particular its richness in C-glycosyl-flavones, as highlighted in previous studies [24-25].



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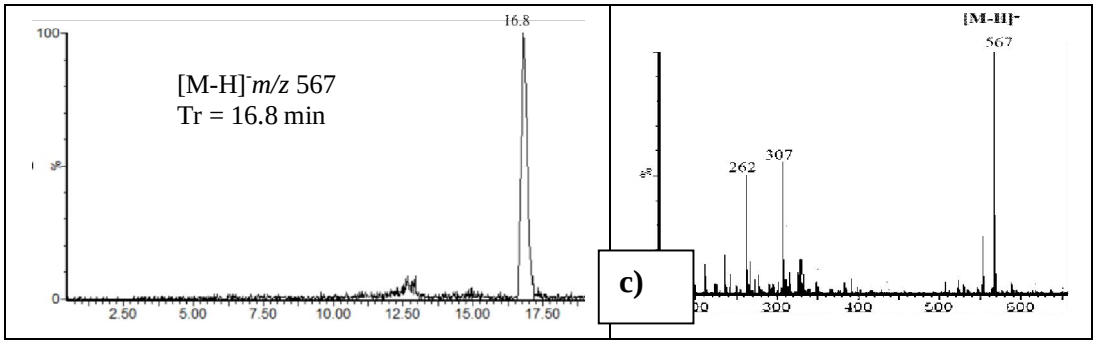

Fig.2.TIC and chromatograms of major peaks analysed and their mass spectra

3.4. Antioxidant activity

DPPH, ABTS and FRAP tests were carried out to assess the antioxidant activity of ECN extract. The results are presented in Table (2). Using the DPPH method, the observed DPPH radical inhibition concentration 50% (IC_{50}) is 0.027 ± 0.006 mg/mL and 0.015 ± 0.001 mg/mL for ECN and trolox respectively. Analysis of the results shows that the antioxidant potential of ECN is non-negligible compared with trolox, and the trend is confirmed by the ABTS and FRAP methods. This antioxidant potential is linked to the extract's content of flavonoids, known for their powerful antioxidant properties. These contents are lower than those of *C. citratus*[14]. Hydrodistillation residues of *C. ladanifer*, *L. intermedia* and *S. rosmarinifolia* showed lower antioxidant activities than ECN extract in this study with IC_{50} of 0.973 mg/mL, 0.815 mg/mL and 0.415 mg/mL respectively [29].

Table 2. Antioxidant activity of ECN extract by DPPH, ABTS and FRAP methods

Extrait	DPPH (IC_{50})(mg/mL)	ABTS (IC_{50})(mg/mL)	FRAP (mg TE/ g of dry extract)
ECN	0.027 ± 0.006	0.35 ± 0.02	263.01 ± 22.62
Trolox	0.015 ± 0.001	0.27 ± 3.42	

3.5. Antibacterial activity, MIC and MBC of the extract

In vitro tests were carried out to assess the antibacterial power of ECN residue extract. The results are presented in Table (3). Zones of inhibition ranged from 9.33 to 10.33 mm for *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC14028, *Escherichia coli* ATCC 35218 and *Staphylococcus saprophyticus* ATCC 2593 at 100 mg/mL compared with the control (Table 3).Chloramphenicol, taken as the reference antibiotic, shows very high inhibition zones, far superior to those of ECN extract at a dose of 30 µg. The activities observed can be considered average. However, the most sensitive bacteria was*Staphylococcus saprophyticus* ATCC 2593 with an inhibition zone of 10.33 ± 1.15 mm, and the least sensitive was *Staphylococcus aureus* ATCC 6538 with an inhibition zone of 9.33 ± 0.57 mm.The ECN extract was therefore inactive on *Pseudomonas aeruginosa* ATCC 9027 and *Klebsiella pneumoniae* strains. *Pseudomonas aeruginosa* is known for its virulence and multiresistance to antibiotics [30].In response to treatment with ECN, the lowest MIC value of 3.12 mg/mL was obtained with *S. aureus* ATCC 6538 and *S. saprophyticus* ATCC 2593. The lowest MIC (12.5 mg/mL) of the ECN extract was obtained with *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC14028 and *Staphylococcus saprophyticus* ATCC 2593 (Table 3). *Escherichia coli* ATCC 35218 remains less sensitive, with aMBC of 25 mg/mL.MBC / MIC ratios are less than or equal to 4 (MBC / MIC ≤ 4), showing that the extract has a bactericidal effect on the strains studied. The activity of *C. nardus* ethanolic extract (ECN) is probably correlated with its total flavonoid content. The presence of flavonoids, and specifically

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flavones such as vitexin and orientin, may account for the antioxidant power of the extract [24].In fact, these two molecules are moderately active, but present in the *Ocimum sanctum* plant they are cited for their synergistic action in inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Klebsiella pneumoniae* and Proteus sp. However, antibacterial activity may be due to the presence of other organic families revealed here by phytochemical screening, such as triterpenes and coumarins [31].

Table 3. Diameters of the inhibition zones of the extracts in relation to the microbial strains studied

		Diameters of the inhibition zones (mm)	
Bacterial strains	**Gram**	**ECN**	**Chloramphénicol (30µg)**
Escherichia coli ATCC 35218	-	10.33± 1.53	25.33 ± 0.58
*Pseudomonas areruginosa*ATCC 9027	-	< 8	20.00 ± 1.00
Klebsiella pneumoniae	-	< 8	24 ± 0.00
Salmonella typhimurium ATCC14028	-	09.66 ±0.57	21.66 ± 1.53
Staphylococcus aureus ATCC 6538	+	0933± 0.57	23.33 ± 0.58
Staphylococcus saprophyticus ATCC 2593	+	10.33 ±1.15	25.66 ± 1.15

Values are expressed as mean of three trials ± standard deviation; < 8: no activity. - means not determined

Table 4. Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of ECN extract

Bacterial strains	**Gram**	**MIC (mg/mL)**	**MBC(mg/mL)**	**MBC/MIC**
E. coli ATCC 35218	-	6.25	25	4
S. typhimurium ATCC14028	-	6.25	12.5	2
S. aureus ATCC 6538	+	3.12	12.5	4
S. saprophyticus ATCC 3518	+	3.12	12.5	4

All experimental measurements were carried out in triplicate. - : means not determined

4. CONCLUSION

The present work has identified several families of secondary metabolites such as tannins, flavonoids, triterpenes and coumarins in the ethanolic extract of *Cymbopogon nardus*hydrodistillation residues. The results also showed that these residues were rich in total polyphenols and total flavonoids. The ethanolic extract of C. nardus showed antibacterial activity against certain strains, notably *S. aureus* ATCC 6538, *S. typhimurium* ATCC14028, *E. coli* ATCC 35218 and *S. saprophyticus* ATCC 2593. The results indicate that *C. nardus*hydrodistillation residues can be exploited as a potential source of bioactive molecules for crop protection.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts

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