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Antioxidants activities, HPTLC and HPLC/MS characterization of some phenolic acids of *Grangea maderaspatana* roots extracts from Burkina Faso

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ABSTRACT

Grangea maderaspatana belonging to Asteraceae family is used for its pharmacological properties. The aim of this study is a bio-guided antioxidant compounds characterization of *Grangea maderaspatana* roots extracts. Extracts were obtained using hexane, dichloromethane, ethyl acetate and methanol. Each crude extract was tested for its free radical scavenging activity using DPPH method. Ethyl acetate extract was found to have the most antioxydant activity with IC_{50}=64.720 µg/mL. This active extract was passed through to column chromatography fractionation to yield seven sub-fractions of which sub-fraction F'"6 was the most active (IC_{50}=2.897µg/mL). HPTLC and HPLC/MS analysis showed that this sub-fraction mainly contained phenolic acids of which eight were identified as chlorogenic acid (5-caffeoylquinic acid), 1,3,5-tricaffeoyl-4-succinoylquinic acid, 3,4,5-tricaffeoylquinic acid, 1,5-dicaffeoyl-4-succinoylquinic acid, 1,3-dicaffeoyl-5-fumaroylquinic acid, 3,5-dicaffeoyl-1-fumaroylquinic acid, 1,5-dicaffeoyl-4-fumaroylquinic acid and 1-fumaroyl-3,5-dicaffeoyl-4-succinoylquinic acid. Thus, it can be seen from the present study that *Grangea maderaspatana* roots extract mainly contain phenolic acids and could be used as natural oxidant.

Keywords: *Grangea maderaspatana*, Free Radical Scavenging Activity, HPTLC, HPLC/MS, Phenolic Acids.

INTRODUCTION

Plants produce organic substances known as secondary metabolites. These compounds have a variety functions (Nebié et al., 2023; Miwonouko et al., 2024). Secondary metabolites are known for their

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pharmacological properties. Most are sought for their antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, anti-malarial and other activities (Nebié et al., 2023; Mensah et al., 2023; Dermane et al., 2024). Thus, bioactive compounds derived from plants are increasingly sought as an alternative to synthetic molecules. It is estimated that around 60% of pharmaceutical products are based on and/or derived from natural products, mainly of plant origin (Quetin-Leclercq, 2002). In Burkina Faso, many medicinal plants are used to treat pathologies. These include *Grangea maderaspatana* (Asteraceae), which is used by local people for its pharmacological properties. This aromatic plant is traditionally used to treat eyes, ears, muscles and joints (Kishore et al., 2015), headaches pains, paralysis and hepatitis (Rachchh et al., 2015). The plant's roots are used as aperitif, intestinal astringent, diuretic, vermifuge, emmenagogue and galactagogue (Kishore et al., 2015; Dhal et al., 2015). Previous studies reported that *G. maderaspatana* has analgesic, diuretic (Ahmed et al., 2001), hepatoprotective (Rachchh et al., 2015), anti-inflammatory, anti-rheumatic (Rachchh et al., 2015; Chang et al., 2016), anti-anxiety, anti-breast cancer (Chimplee et al., 2019), antioxidant and antimicrobial properties (Darshan et al., 2013; Dabiré, 2011). Recent studies have also showed that the plant's flowers, roots and leafy branches are rich in phenolic compounds, flavonoids, alkaloids, sterols and terpenes (Yougoubo et al., 2021). Some authors have also revealed that leafy branches have anti-plasmodial properties (Yougoubo et al., 2024). To our knowledge, few studies have been reported on antioxidant potential of bioactive compounds in the plant's roots. Thus, the aim of this study was to characterise antioxidant compounds in roots of *G. maderaspatana* collected in Burkina Faso.

MATERIALS AND METHODS

Plant material

Plant material consists of *G. maderaspatana* roots. The whole plant was harvested in Ouagadougou, precisely in the vicinity of dam of the said town (12°23'32.1"N; 1°32'33.03"W). Roots were separated from whole plant and dried in laboratory before being ground into powder (Figure 1).

Preparation of extracts

Extracts were obtained by successive exhaustion using solvents of increasing polarity: n-hexane, dichloromethane, ethyl acetate and methanol. 100 g of roots powder were macerated with 500 mL of each solvent. All extractions were repeated in triplicate. The filtrates were combined and concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 35ºC.

Anti-free radical activity assessment

Antiradical activities of extracts and fractions were assessed using radical DPPH· method as described by Toure et al. (2023). This protocol consists of adding 200 µL (10^{-4} mol.L^{-1}) of radical DPPH· solution to 50 µL of each extract prepared at different concentrations. The mixture was shaken and kept at room temperature protected from light for 10 min. Absorbances were read at 517 nm using a microplate spectrophotometer (MP96 spectrophotometer, SAFAS). Inhibition and decolourisation of the DPPH· radical depended on the concentration of the extracts used. IC_{50} of each extract and standards, expressed in µg/mL, were calculated graphically by linear regressions of the plotted graphs, by relating half absorbance of radical DPPH· without sample to the regression curve of sample. Antioxidant activity of fractions obtained was expressed by the antioxidant activity index (AAI), calculated as follows: AAI=(DPPH concentration)/IC_{50}

Based on AAI, a scale proposed by Scherer et al. (2009) was used:

- For AAI<0.5, low antioxidant activity
- For 0.5<AAI<1, moderate antioxidant activity
- For 1<AAI<2, high antioxidant activity
- For AAI>2, very high antioxidant activity.

Fractionation by column chromatography

Extract with highest anti-free radical activity was fractionated on a column. 2 g of this root extract was fractionated on a 49 cm long column. Filled silica gel (Fluka chemika Silica gel 60, 63 mm, 230-400 mesh) as stationary phase. Eluents used were the following solvent systems: n-hexane; n-hexane-ethyl acetate; ethyl acetate-methanol and ethyl acetate-formic acid-water in variable proportions. Fractions were collected in 15 mL falcon tubes. A total of 28 fractions were obtained. Thin Layer Chromatography (TLC) of fractions obtained was carried out using migration system: ethyl acetate-formic acid-water (80:10:10) and detected at UV/366 nm after spraying with NEU's reagent. On the basis of similar TLC profiles, fractions were gathered. All fractions were concentrated to minimum solvent using a rotary evaporator, then transferred to 2 mL Eppendorf tubes and concentrated to dryness in a vacuum pump. All the fractions were tested for their antioxidant activity.

High performance thin layer chromatography (HPTLC)

The most active fraction was chosen for further work. HPTLC of this fraction and some standards was carried out and visualised under UV/366 nm after derivatisation with Neu's reagent. These standards included quercetin, rutin, chlorogenic acid, caffeic acid, rosmanic acid, ferulic acid and gallic acid. CAMAG HPTLC system equipped with an Automatic TLC Sampler4 (ATS4), an Automatic Developing Chamber (ADC2) and a TLC scanner was used to carry out this analysis. VisionCATS integration software was used for data acquisition. The procedure consisted of automatically depositing 10 µL of each compound onto a silica gel 60 F254 HPTLC plate (20x10 cm) with a width of 8 mm. After deposition, the plate was saturated in ADC2 for 20 min. After development with EtOH, DCM, H_2O, HCOOH system (16:16:4:1, v/v), the plate was dried for 5 min in ADC2 and visualised in visible, UV/254 and 366 nm using TLC visualizer 2. The plate was then derivatized with NEU/PEG reagent using CAMAG derivatizer and placed on a hot plate at 120°C for 5 min. Finally, the plate was then visualised again in the visible, at UV/254 and 366 nm.

High-performance liquid chromatography and mass spectrometry

The most active fraction of *G. maderaspatana* roots was characterised by high-performance chromatography-mass spectrometry. An Agilent Technologies type 1260 Infinity II HPLC was used. It was fitted with a G711A pump, a G7129 automatic injector and a G7121 UV detector. UV-DAD (Diode Array Dectection) detector records in the range 200 to 600 nm. 25 µL of the sample prepared at 1 mg/mL is automatically injected using a syringe maintained at 15°C. Compounds were separated on a Phenomenex C18 column (250 x 4.6 mm; 5 µm) maintained at 35°C. The mobile phase consisted of two solvents A and B at a flow rate of 1 mL/min. Solvent A was water-formic acid 0.1% and solvent B was acetonitrile. Gradient was started with an initial composition of 95% solvent A and 5% solvent B for 1 min. It was then maintained at 74% A and 26% B between 1 and 60 min. Between 60 and 65 min, the elution was changed to 100% B and to 95% A and 5% B between 65 and 67 min. The wavelength used was 254 nm.

Mass spectra of compounds were obtained using Expression CMS Advion mass spectrometer with electrospray ionisation source in negative mode. Various fragmentations in 20 to 120 V range were applied. This spectrograph was fitted with a capillary set at 2500 V. The gas flow rate (N_2) was 7 L/min at 200°C. The scanning range was 50 to 1000 m/z. Spectral data was provided by Advion Mass Express software.

Statistical analysis

IBM SPSS Statistics version 25.0 using ANOVA channel for multiple comparisons was used for statistical analysis of data. The experiments on the assays were triplicated. Results were expressed as mean ± standard deviation and $p<0.05$ values were considered statistically significant.

Grangea maderaspatana (a) **Roots powder (b)**

Figure 1: *Grangea maderaspatana* (a) and its roots powder (b)

RESULTS

Antioxidant potential of extracts

Anti-free radical activity of *G. maderaspatana* extracts and some standards antioxidants was expressed as IC_{50} (inhibitory concentrations) values obtained using the regression curves. IC_{50} values (µg/mL) for the different extracts are summarised in Table 1.

Fractionation of active extracts and assessment of their antioxidant potential

Ethyl acetate extract of *G. maderaspatana* roots which showed the best antioxydant activity was then subjected to column fractionation using solvent systems in varying proportions. Several fractions were collected and gathered to seven fractions based on TLC analysis as presented in Table 2.

HPTLC data analysis of fraction F'''6 and some standards

Results of HPTLC analysis are presented in Table 4. The standards, including chlorogenic acid, caffeic acid, rosmarinic acid, ferulic acid and gallic acid, exhibited blue fluorescence (Figure 2) with reference values of 0.53, 0.90, 0.84, 0.92 and 0.83, respectively. Standards such as quercetin and rutin displayed yellow fluorescence, with the following values: 0.92 and 0.54, respectively. Fraction F'"6 exhibits three distinct spots of blue fluorescence at varying frontal references (0.53, 0.74 and 0.89).

HPLC/MS data for fraction F'''6

Chromatogram of fraction F'"6 showed twenty-three (23) signals numered **1** ; **2** ; **3** ;**4** ; **5** ; **6** ; **7** ; **8** ; **9** ; **10** ; **11** ; **12** ; **13** ; **14** ; **15** ; **16** ; **17** ; **18** ; **19** ; **20** ; **21** ; **22** ; **23** (Figure 3) of which eight (08) were identified on the basis of their UV-visible and mass spectra.

Identification of compound 6 at retention time $t_{R=}$ 19.723 min

Compound **6** was identified based on its UV-visible spectra (Figure 4) and its mass spectra (Figure 5). Analysis of UV-visible spectra of compound **6** shows two maximum absorption bands (Figure 4). The first band is located at 220 nm with a shoulder around 244 nm and the second band is at 330 nm with a shoulder around 296 nm. The mass spectra analysis of compound **6** has yielded the identification of characteristic peaks at m/z 352.9, 190.8, 178.9 and 134.7.

Table 1: IC_{50} values of standards and *G. maderaspatana* roots extracts.

	Standards/Extraits	R^2	Equation of line	IC_{50} (µg/mL)
Standards	Trolox	0.9988	y=-0.0137x + 0.6002	14.747±0.339[a]
	Quercétin	0.9978	y=-0.0259x + 0.5887	7.440±0.058[a]
	Gallic acid	0.9912	y=-0.1257x + 0.5905	1.510±0.007[a]
	Rutin	0.9982	y=-0.0055x + 0.6045	38.485±0.367[a]
	Ascorbic acid	0,9973	y =-0.0189x + 0.6028	11.100±0.216[a]
Roots extracts	Hexane	nd	nd	nd
	DCM	0.9872	y=-0.0709x + 0.5674	6553.333±76.376[d]
	EtOAc	0.9984	y=-0.0031x + 0.5598	64.720±0.566[ab]
	MeOH	0.9963	y=-9E-05x + 0.5806	2252.963±76.645[c]

nd : not detected, **DCM** : Dichloromethane, EtOAc : Ethyl acetate, **MeOH** : Methanol
Values are expressed as mean ± standard deviation. Means in each column followed by a different letter are significantly different (P<0.05).

Table 2: Fractionation of ethyl acetate extracts.

EtOAc crude extract	Fractions	Mobile phase	Mobile phase composition	Solvent volume (mL)	Fraction mass (mg)
Roots	F'"1	Hex-EtOAc	100:00 to 60:40	100x3	410.5
	F'"2	Hex-EtOAc	20:80 to 00:100	100x3	31.2
	F'"3	EtOAc-MeOH	80:20	100x2	26.2
	F'"4	EtOAc-MeOH	50:50	100x1	13.7
	F'"5	EtOAc-MeOH	00:100	100x2	20.2
	F'"6	EtOAc-HCOOH-H_2O	80:10:10	100x2	250
	F'"7	EtOAc-HCOOH-H_2O	80:10:10	100x1	113.3

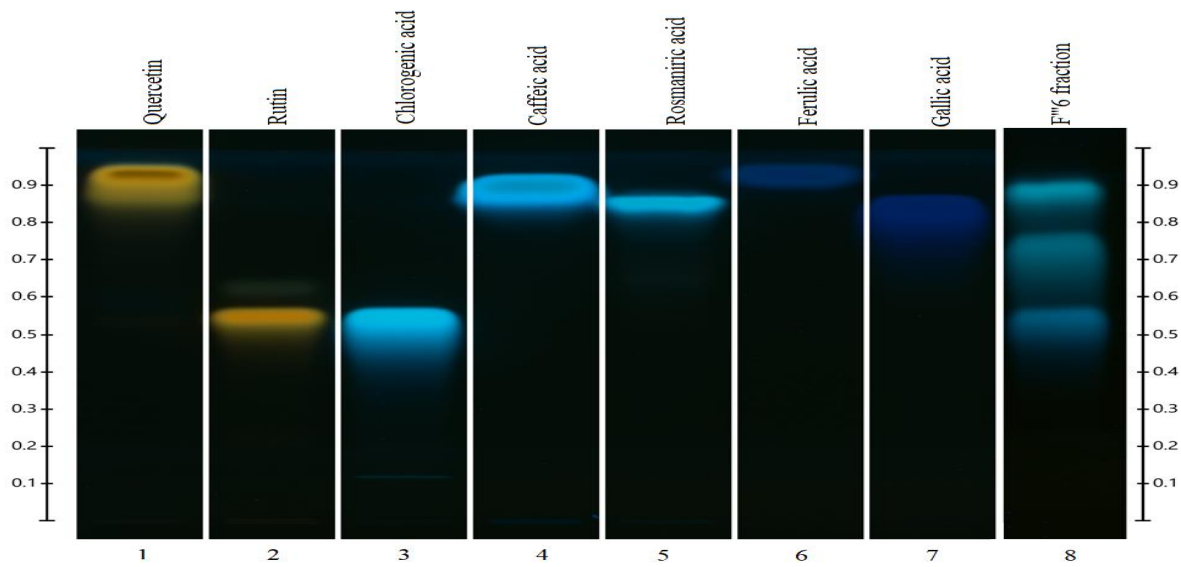


Figure 2: Chromatogram of fraction F'"6 and some standards analysed by HPTLC.

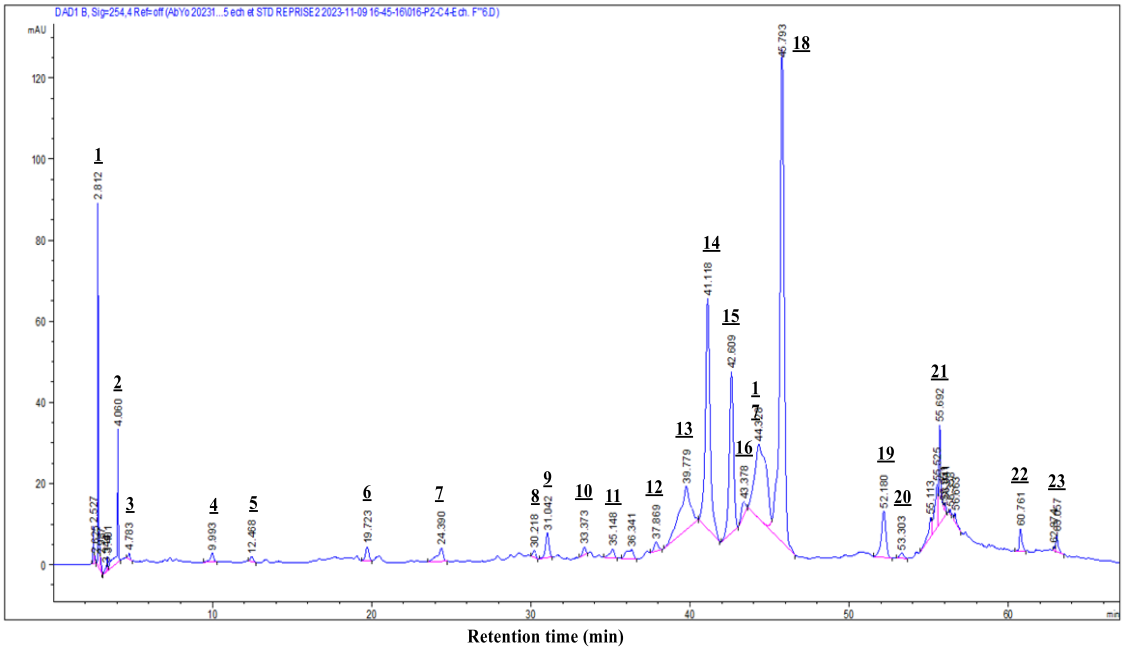


Figure 3: HPLC Chromatogram of fraction F'"6.

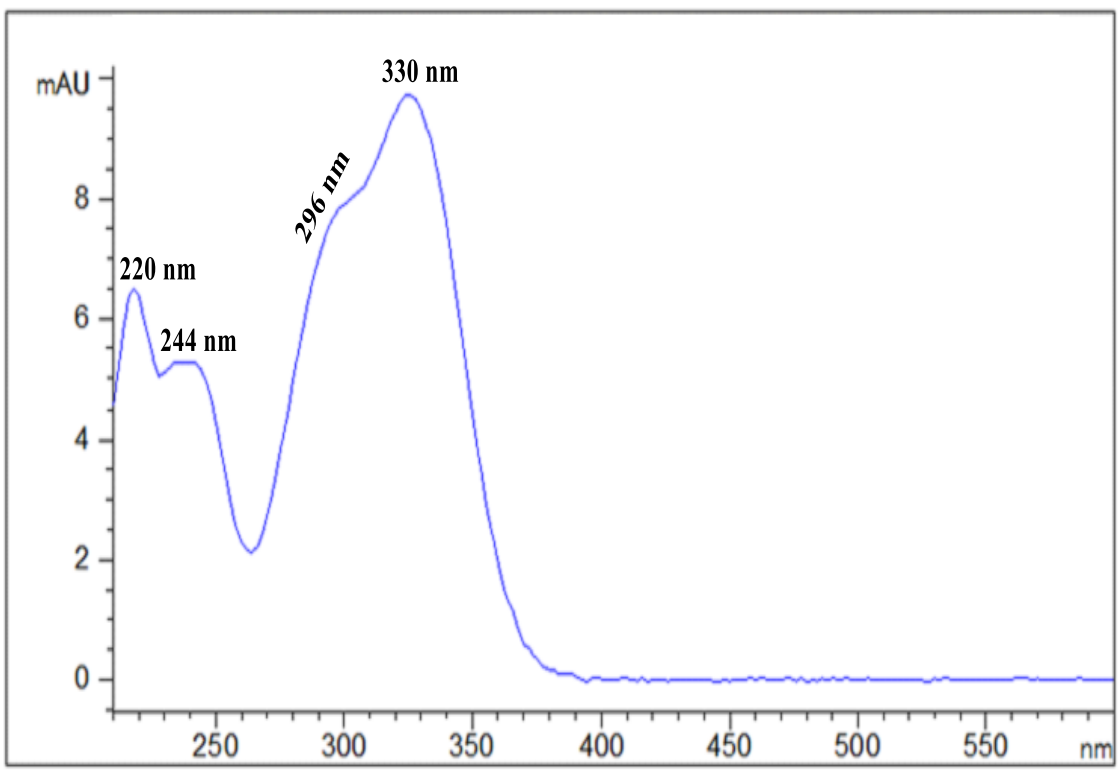


Figure 4: UV spectra of compound **6.**

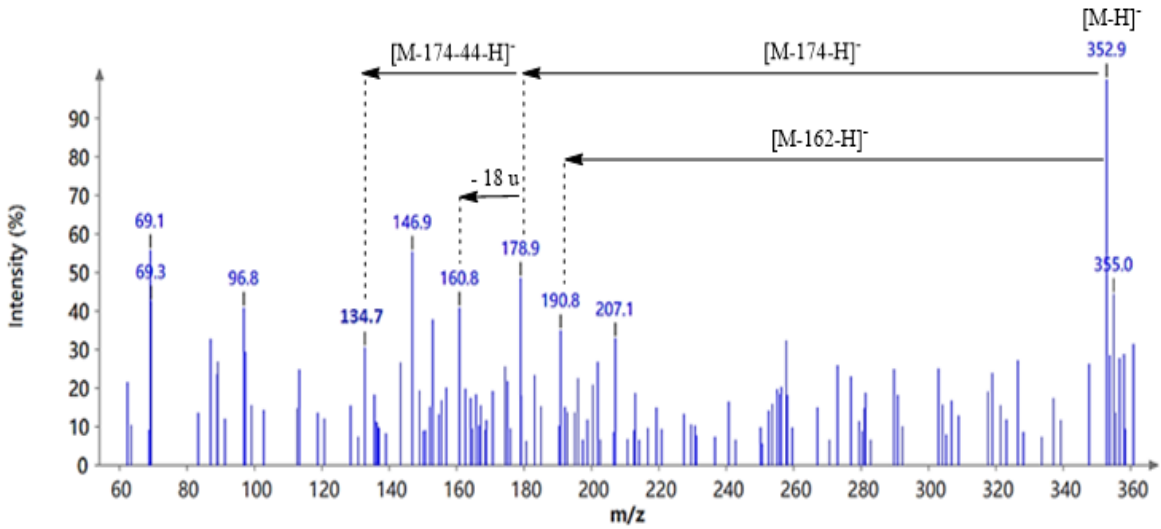


Figure 5: Mass spectra of compound **6.**

DISCUSSION

Crude extract IC_{50} values range from 64.720 for EtOAc extract to 6553.333 µg/mL for DCM extract. It is known that the lower is IC_{50} value of extract, the higher is free radical scavenging capacity of th

e extract is higher (Varsha et al., 2015 ; Wang et al., 2019). As a result, Ethyl acetate extract is the most active. Ethyl acetate should be the most suitable solvent for extracting antioxidant compounds from *G. maderaspatana* roots.

Antioxidant potential of the fractions was determined and was expressed as IC_{50} and antioxidant activity index (AAI) values. Results are presented in Table 3. Statistical analysis reveals that the inhibitory concentrations (IC_{50}) and index (AAI) obtained are significant *($P<0.05$)* and vary from one fraction to another. AAI ranged from 0.114 for fraction F'"1 to 13.807 for fraction F'"6. Thus, using scale proposed by (Scherer et al., 2009), fractions obtained from roots have very high antioxidant activity except for fraction F'"1 whose activity is low. All fractions obtained showed higher activity than that of crude extract. Similar observations were reported by (Dabiré et al., 2015). Indeed, after column chromatography of ethanolic extract of *Lippia multiflora*, these authors found that one of fractions showed higher antioxidant activity (IC_{50}=23.68µg/mL) than that of crude extract (IC_{50}=38.20µg/mL). According to literature (Kishore et al., 2015 ; Varsha et al., 2015 ; Dodiya et al., 2017a ; Dodiya et al., 2017b), *Grangea maderaspatana* roots have traditionally been used as an aperitif and as an intestinal astringent with diuretic, vermifuge, emmenagogic and galactagogic properties. These various properties attributed to the plant's roots could be due to the very high antioxidant activity observed. Fraction F'"6 could be used to characterise major compounds in roots extracts. To our knowledge, very little interest has been shown in bioactive compounds of *G. maderaspatana* roots. Previous work have focused mainly on leaves and the whole plant extracts (Madhava et al., 2009 ; Kishore et al., 2015 ; Chang et al., 2016). HPTLC analysis presented in Table 4 show three main spots with different frontal references in fraction F'"6. Light blue in fluorescence, fraction F'"6 spots have frontal references of 0.53, 0.74 and 0.89 (Figure 2). The basis of the fluorescence and frontal reference values of the various compounds could be that fraction F'"6 contain chlorogenic acid, caffeic acid and/or derivatives of these

compounds (Figure 2). Compounds contained in fraction F'"6 are essentially phenolic acids.

The chromatogram of fraction F'"6 showed twenty-three (23) of which eight (08) were identified. These were signals **6**, **13**, **14**, **15**, **16**, **17**, **18** and **19** (Figure 3). Analysis of mass spectrometry related to these signals shows that they are essentially phenolic acids, particularly chlorogenic acid and derivatives. This is in agreement with data from HPTLC analysis (Figure 2). Several pharmacological activities such as antioxydant, antispasmodic and anticancer have been attributed to chlorogenic acid and its derivatives (Rakesh et al., 2011). The presence of chlorogenic acid and its derivatives could contribute to the higher antioxidant activity observed with the ethyl acetate extract of roots in general and fraction F'"6 in particular. Also, fraction F'"6 is found to be more potent (IC_{50} = 2.89 µg/mL) than standards such as trolox (IC_{50} = 14.74 µg/mL), quercetin (IC_{50} = 7.44 µg/mL), rutin (38,48 µg/mL) and ascorbic (IC_{50} = 11.10 µg/mL).

According to literature, the band at 220 nm and the shoulder at 296 nm are due to carbonyl group of compound, while aromatic group is responsible for the band observed at 330 nm and the shoulder at 244 nm (Navarra et al., 2017 ; Catauro et al., 2020). This UV spectra is characteristic of chlorogenic acid or one of its derivatives (Michael et al., 2006 ; Velkoska-Markovska et al., 2019 ; Wang et al., 2019). Analysis of mass spectra of compound **6** revealed peaks at m/z 352.9, 190.8, 178.9 and 134.7. The peak at m/z 352.9 $[M-H]^-$ could correspond to the molecular ion of compound **6** (Figure 5). This peak corresponds to the molecular formula $C_{16}H_{17}O_9$. Ion fragment at m/z 190.8 $[M-162-H]^-$ corresponds to a loss of 162 u of molecular ion. The loss of 162 u would be caffeoyl. This fragment could be $[quinic\ acid-H]^-$. Ion fragment at m/z 178.9 $[M-174-H]^-$ corresponds to the loss of 174 u of molecular ion corresponding to loss of quinyl. This peak would be $[caffeic\ acid-H]^-$. The peak at m/z 134.7 $[M-174-CO_2-H]^-$ could come from $[caffeic\ acid-H]^-$ decarboxylation. The peak at m/z 160.8 could correspond to $[caffeic\ acid-H]^-$ dehydration (figure 6). These data are consistent with those of the literature (Rakesh et al., 2011) and show that compound **6** should be chlorogenic acid (5-caffeoylquinic acid) (Figure 7*)*.

Characterisation of chlorogenic acid by HPLC/MS in this fraction is in agreement with the HPTLC analysis. HPTLC analysis of the fraction showed a spot colour and frontal reference similar to those of chlorogenic acid (Figure 2). This molecule is known for its antioxidant, antiviral, antibacterial and antifungal properties (Jassim et al., 2003). Compounds **13**, **14**, **15**, **16**, **17**, **18** and **19** were also identified based on their UV-visible and mass spectra. All the results obtained are presented in Table 5 and structures of different compounds are presented in figure 8.

Table 3: IC_{50} and AAI Values of fractions of ethyl acetate crude extract.

Ethyl acetate crude extract	Fractions	R^2	Equation of line	IC_{50} (µg/mL)	AAI
Roots	F'"1	0.9945	y=-0.0005x + 0.5367	349.4 ± 6^{d}	0.114 ± 0.002^{a}
	F'"2	0.9946	y=-0.0236x + 0.5513	8.021 ± 0.127^{abc}	4.987 ± 0.079^{d}
	F'"3	0.9970	y=-0.0291x + 0.5772	7.395 ± 0.103^{abc}	5.409 ± 0.075^{e}
	F'"4	0.9927	y=-0.0287x + 0.7474	13.428 ± 0.104^{c}	2.979 ± 0.023^{b}
	F'"5	0.9924	y=-0.0373x + 0.7289	9.836 ± 0.080^{bc}	4.067 ± 0.033^{c}
	F'"6	0.9894	y=-0.0709x + 0.5674	2.897 ± 0.042^{a}	13.807 ± 0.202^{g}
	F'"7	0.9990	y=-0.0441x + 0.6068	5.551 ± 0.068^{bc}	7.206 ± 0.088^{f}

Values are expressed as mean ± standard deviation. Means in each column followed by a different letter are significantly different ($P < 0.05$).

Table 4: Frontal reference of HPTLC analysis of fraction F'"6 and standards.

Number	Standards and fraction	Fluorescence	Rf
1	Quercetin	Yellow	0.92
2	Rutin	Yellow	0.54
3	Chlorogénic acid	Light blue	**0.53**
4	Caffeic acid	Light blue	**0.90**
5	Rosmaniric acid	Light blue	0.84
6	Ferulic acid	Dark blue	0.92
7	Gallic acid	Dark blue	0.83
8	Fraction F'''6	Light blue	**0.53**
		Light blue	0.74
		Light blue	**0.89**

Table 5: Compounds identified in fraction F'"6.

Peak number	Rt(min)	λ (nm)	$[M-H]^-$	Characteristic fragment ions	Formula	Compound name
6	19.723	220; 244 (sh); 296 (sh); 330	352.9	190.8 ; 178.9 ; 160.8 ; 134.7	$C_{16}H_{17}O_9$	chlorogenic acid
13	39.779	218 ; 246 (sh) ; 298 (sh) ; 328	776.7	514.8 ; 453.0	$C_{38}H_{33}O_{18}$	1,3,5-tricaffeoyl-4-succinoylquinic acid
14	41.117	220 ; 246 (sh) ; 296 (sh) ; 330	676.7	515.0 ; 352.7 ; 335.1	$C_{34}H_{29}O_{15}$	3,4,5-tricaffeoylquinic acid
15	42.609	220 ; 246 (sh) ; 296 (sh) ; 330	614.6	515.0 ; 453.4 ; 352.9 ; 335.1	$C_{29}H_{27}O_{15}$	1,5-dicaffeoyl-4-succinoylquinic acid
16	43.378	220 ; 246 (sh) ; 292 (sh) ; 326	612.9	567.8 ; 515.1 ; 353.3	$C_{29}H_{27}O_{15}$	1,3-dicaffeoyl-5-fumaroylquinic acid
17	44.328	220 ; 246 (sh) ; 296 (sh) ; 330	612.7	514.9 ; 353.1 ; 190.1 ; 179.0	$C_{29}H_{25}O_{15}$	3,5-dicaffeoyl-1-fumaroylquinic acid
18	45.793	220 ; 246 (sh) ; 296 (sh) ; 330	612.7	515.0 ; 451.5 ; 353.2	$C_{29}H_{25}O_{15}$	1,5-dicaffeoyl-4-fumaroylquinic acid
19	52.180	218 ; 246 (sh) ; 298 (sh) ; 328	712.6	613.0 ; 514.8 ; 435.2 ; 352.9 ; 190.6 ; 178.9	$C_{33}H_{29}O_{18}$	1-fumaroyl-3,5-dicaffeoyl-4-succinoylquinic acid

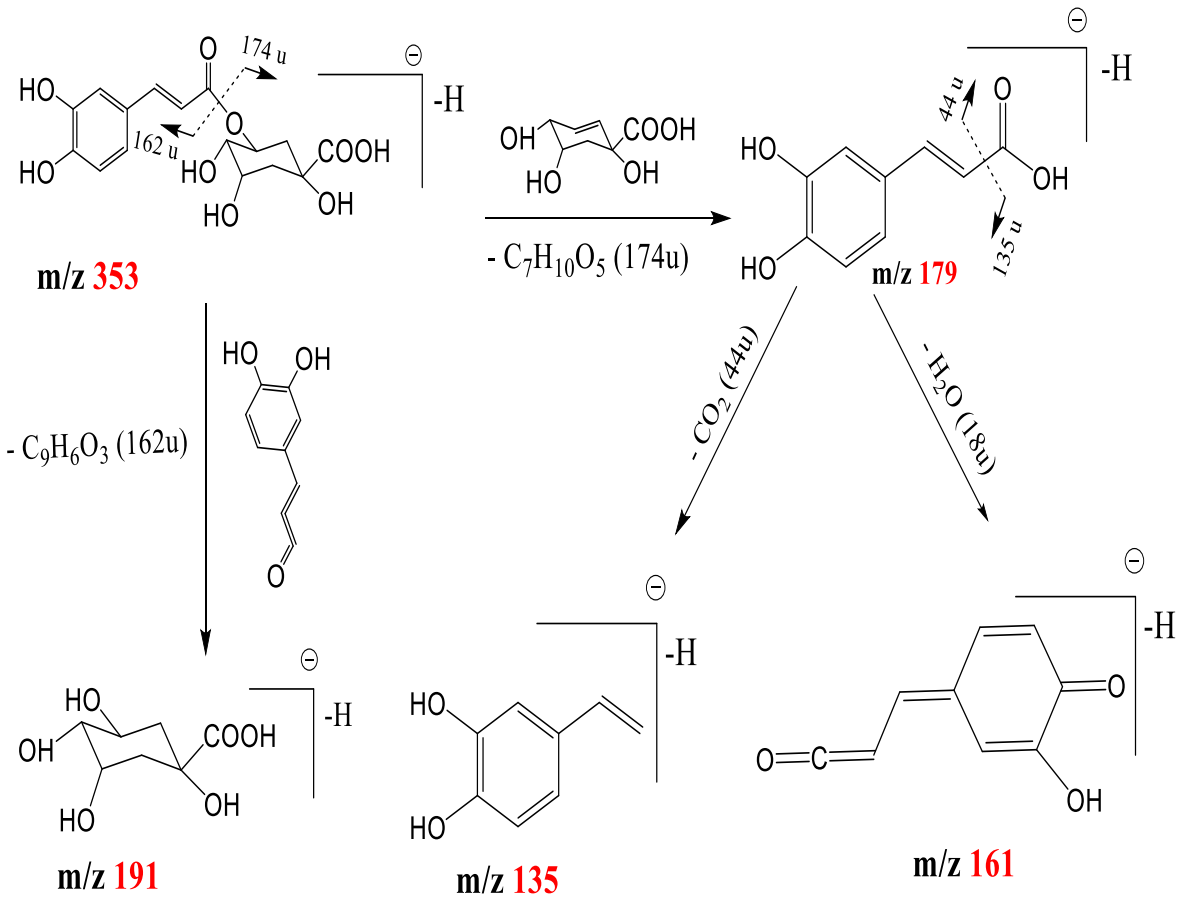
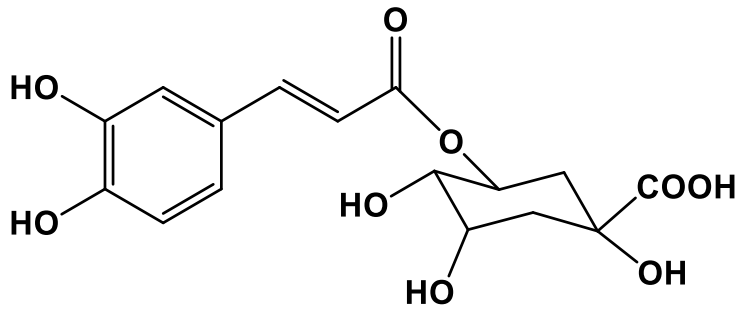


Figure 6: Fragmentation mechanism of compound **6.**



Chlorogenic acid (5-caffeoylquinic acid)

Figure 7: Structure of compound **6.**

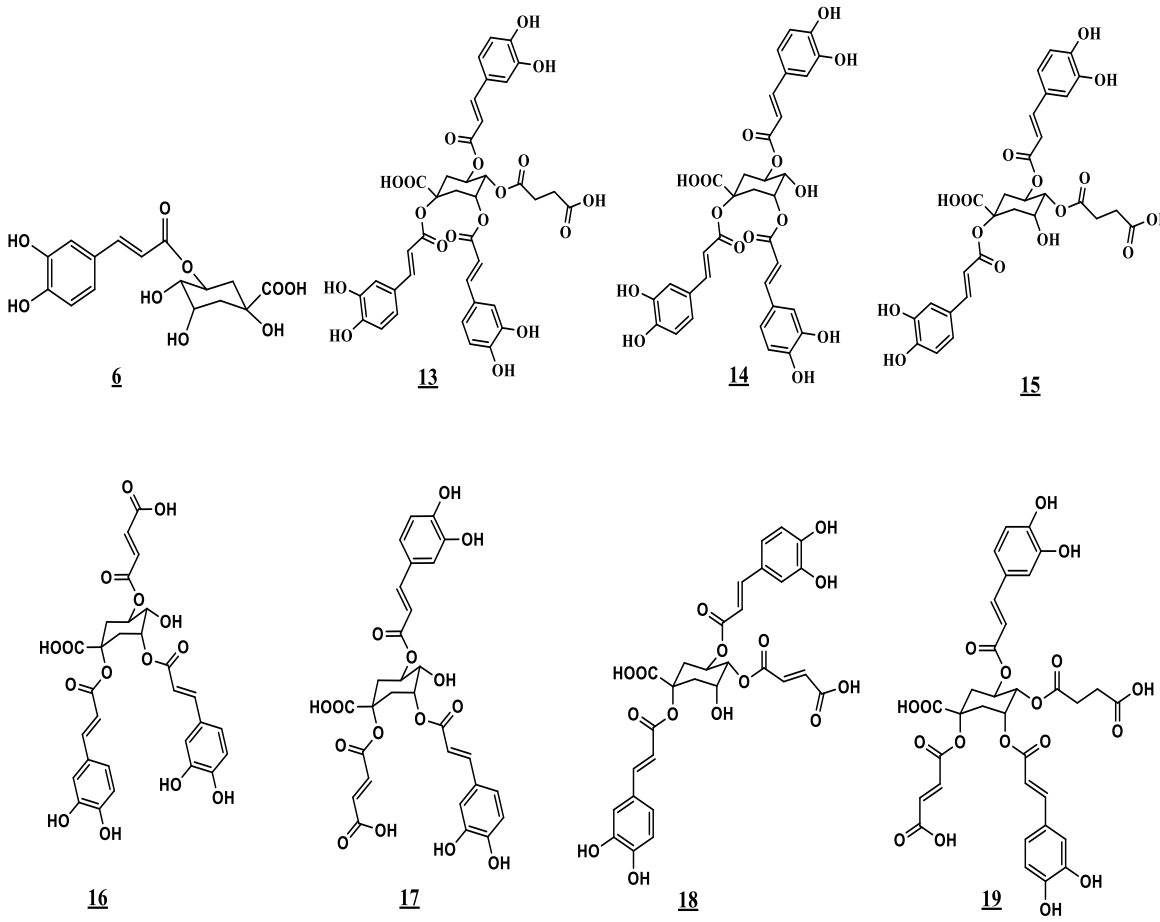


Figure 8: Structures of compounds.

Conclusion

Grangea maderaspatana roots harvested in Burkina Faso are rich in bioactive substances especially phenolic acids. Using HPLTC and HPLC/MS techniques, these phénolic acids have been identified as chlorogenic acid, 1,3,5-tricafeoyl-4-succinoylquinic acid, 3,4,5-tricaffeoylquinic acid, 1,5-dicaffeoyl-4-succinoylquinic acid, 1,3-dicaffeoyl-5-fumaroylquinic acid, 3,5-dicaffeoyl-1-fumaroylquinic acid, 1,5-dicaffeoyl-4-fumaroylquinic acid and 1-fumaroyl-3,5-dicaffeoyl-4-succinoylquinic acid. The presence of these compounds in roots could justify traditional use.

COMPETING INTERESTS

Authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

AY, MCD and KRB designed and directed the study. AY conducted the experiments. All authors contributed to the writing of the document.

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