Chemical Composition, Antioxidant and Antibacterial Activities of *Brocchia cinerea* From South-East of Morocco

Aicha Hamdouch[1], Said Gharby[2*], Ali Asdadi[1], Hasnae Ait Bouzid[2], Fouad Achemchem[3], Bouchra Chebli[4], Lalla Mina Idrissi Hassani[1]

[1]*Laboratory of Plant biotechnology, Faculty of science, IBN ZOHR University, Agadir Morocco*

[2]*Laboratory of Biotechnology, Materials and Environment, Polydisciplinary Faculty of Taroudant, IBN ZOHR University, Agadir Morocco*

[3]*Laboratory of Biology, Superior School of Technology, Faculty of science, IBN ZOHR University, Agadir Morocco*

[4]*Laboratory of Mechanics, Processes, Energy and Environment, National School of Applied Sciences, IBN ZOHR University, Agadir Morocco*

Abstract:

The essential oil of wild-growing medicinal specie *Brocchia cinerea* from oasis of Tata (Southeast of Morocco) was studied for chemical composition, antioxydant and antibacterial activities. The essential oil is extracted using a clevenger distillation apparatus. The antioxidant activity was determined by measuring the free radical scavenging power of DPPH and the antibacterial activity by broth dilution method Gas chromatography-mass spectrometry (GC/MS) results revealed that the major compounds of *Brocchia cinerea* are Thujone, Camphor and Eucalyptol. The antibacterial test of *Brochia cinerea* essential oil revealed a strong antibacterial activity against *Listeria innocua, Pseudomonas aeruginosa* and *Staphylococcus aureus* (MIC<1 µL) and also a high scavenging effect of DPPH (IC_{50}=0.080± 0.014).

Keywords: Antibacterial activity, *Brochia cinerea*, Essential oil, DPPH, GC/MS

**Corresponding author:* s.gharby@yahoo.fr/s.gharby@uiz.ac.ma

Introduction:

The oasis of Tata is located in the southeast of Morocco. Geographically, the area is on the southern side of the Anti-Atlas Mountains and occupies an area of 26,274 sq/km. The area is characterized by a hyper aridity with low rainfall of about 100 mm and large variations in daily and annual temperatures. Its population is predominantly rural (70%) (Abouri *et al.*, 2012).

Brocchia cinerea (Del.) Vis. (=*Cotula cinerea* (Del.) Vis.) is a xerophytic plant widely distributed in sandy and desert soils (Fennane *et al.*, 1998). It bears several vernacular names: "*L-gertôfa*" in the Western Sahara; "*Tiklilt, taklilt*" in the oasis of south-east of Morocco. It's used in the treatment of broncho pulmonary diseases, diarrhea and used to flavor of milk and tea (Maiza *et al*., 1996; Bellakhdar, 1997).

This plant contains a various chemical compounds with therapeutic properties, such as flavonoids (Dendougui *et al.*, 2012), terpenes and essential oils (Kasrati 2015; El Abdouni *et al.*, 2016; Bouziane *et al.*, 2013). The presence of terpenes and essential oils gives the plant its olfactory specificity.

The use of natural substances such as essential oils against bacteria and as naturally occurring antioxidants has considerably importance in recent times and much research continues to be undertaken in this direction (Hamdouch *et al.*, 2022, Harhar *et al.*, (2010), Asdadi *et al.*, 2015; Gharby *et al*., 2020; Hajib *et al.*, 2020; Ed-Dra *et al*., 2020).

This study contributes to the valorization of a Saharan species: *Brocchia cinerea* by studying the chemical composition of its essential oil and evaluating its antibacterial and antioxidant activities.

•Materials and Methods:

Plant materials

The study was carried out on essential oil from aerial part (leaves, flowers and steams) of *Brocchia cinerea* growing in a marginalized oasis situated in the Southeast of Morocco. Taxonomic identification of the species was confirmed by Pr. Fouad Msanda, a plant taxonomist at the Laboratory of Plant Biotechnologies of the University Ibn Zohr, Agadir, Morocco. The plant was harvested in May-July 2018 at Tata oasis (Altitude 670 m), Morocco, then, it was

dried in shadow and deposited in the herbarium of the laboratory of plant Biotechnologies, Planta Sud unity, Faculty of Sciences, University Ibn Zohr, Agadir, Morocco.

Essential oil extraction

The essential oil was extracted by hydrodistillation in a Clevenger apparatus according to the European Pharmacopoeia (Ainane *et al.,* 2019). A portion (100 g) of the dried aerial part was subjected to water-distillation, using a Clevenger-type apparatus for 3 hours. The obtained essential oil was dried over anhydrous sodium sulphate; and after filtration, it was stored at + 4°C until use. The yield of the essential oil (%) was calculated as follows (Ed-Dra *et al.,* 2020):

%Yield = (Essential oil (g) /Dry matter (g))*100

Gas Chromatography-Mass Spectrometry (GC / MS) analysis

The essential oil of aerial parts of *Brocchia cinerea* was analyzed by gas chromatography/mass spectrometry (GC/MS) using Shimadzu system. A VB5 column (30m x 0.25mm inner diameter, 0.25 µm film thickness) was used with helium as the carrier gas (1 ml/min). The temperature was held at 50°C for 1 min and programmed at 280°C at a rate of 10°C/min, then kept constant at 280°C for 2 min. Split flow was used; the injector temperature was 250°C. MS were taken at 70 eV at 300°C. The temperature range was 300 °C. The individual compounds were identified by MS. A library search was carried out using the "Wiley GC/MS Library" Nist147 database LIB. Retention indices (RI(exp)) of compounds were determined relative to the retention times of a series of n-alkanes (C_5H_{12}–$C_{24}H_{50}$) and were further confirmed by comparing their retention indices with those reported in the literature (RI(a)) (Gharby *et al.*, 2020; Hajib *et al*., 2020).

Antibacterial activity

The antibacterial activity of essential oil of *Brocchia cinerea* was evaluated against three kinds of bacteria; *Staphylococcus aureus, Listeria innocua* and *Pseudomonas aeruginosa*, using broth microdilution method.

The bacterial suspensions were adjusted with sterile saline solution to a concentration of 10^6 UFC/mL. The inocula were prepared daily and stored at +4°C until use

Broth microdilution method was used to determine the MIC and MBC (NCCLS, 1999; Yu *et al.,* 2004, Deliorman Orhan *et al.,* 2010; Ustun *et al.,* 2016). All tests were performed in Mueller Hinton broth (MHB) supplemented with Tween 80. Serial doubling dilutions of *Brocchia cinerea* essential oils were prepared with concentration ranging from0.2 to 30 µg/mL in MHB.

Every hemolysis tubes was supplemented with 10 µl of the final concentration (10^6 CFU/ml) for each tested bacteria. Inoculated hemolysis tubes were incubated at 37°C for 14 h. The MIC (Minimal Inhibition Concentration) is defined as the lowest concentration of the essential oil at which the microorganisms does not demonstrate visible growth. The microorganisms growth is indicated by the turbidity

The MBCs (Minimal Bactericidal Concentration) were determined by serial sub-cultivation of 10 µL of hemolysis tubes not presenting turbidity onto Mueller Hinton Agar with further incubation for 24 h at 37°C. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum

Determination of DPPH Radical Scavenging Activity

The *in vitro* antioxidant activity of the essential oil of *Brochia cinerea* growing in the Oasis of Morocco was assessed by measuring free radical scavenging activity of DPPH (2,2-diphenyl-1-picryhydrazyl) according to Chen *et al*. (2004) and Leitao *et al*. (2002) with some modifications.

Essential oil of *Brochia cinerea* was prepared at different concentrations (0.1mg/mL, 0.05 mg/mL, 0.025 mg/mL, and 0.0125 mg/mL). 500 µL of each concentration was mixed with 500 µL of a methanol solution of DPPH (0.004 %). After incubation for 30 minutes in the dark at room temperature, the absorbance is measured at 517 nm wave length. The inhibition of the free radical DPPH by butylhydroxytoluene (BHT) and and Covi-ox T_{50} was also analyzed with the same concentrations under the same conditions and had been used as positive control. The inhibition rate of the free radical DPPH (I%) was calculated as follows:

$I\% = 100 \times (A_{control} - A_{test})/A_{control}$ (Chen *et al*., 2004; Leitao *et al*., 2002)

Where $A_{control}$ is the absorbance of the control (containing all reagents without the test product) and A_{test} is the absorbance of the test compound (containing all reagents and the test product). The value IC_{50} is calculated from the graph of the DPPH scavenging effect rate against the sample concentration and it is used to characterize the antioxidant activity of the essential oil. All tests were performed in triplicate for each concentration.

Statistical Analysis

All data were subjected to statistical analysis of variance using STATISTICA software, version 6, Stat-Soft, 2001, France. Newman & Keuls tests were used to segregate treatments which were significantly different ($p< 0.05$).

Results and Discussions

Gas Chromatography-Mass Spectrometry (GC/MS) analysis

The yield of the essential oil from *Brocchia cinerea* aerial parts harvested in the oasis of Tata was 0,66±0,02%/. Previous studies performed on dry aerial parts of *Brocchia cinerea* harvested from other region of Southern Morocco: Alnif, Zagora and Smara showed an extraction yield of 0.55% 0.87% and 0.64%, respectively (Chlif *et al.*, 2021; Ekhlil et al., 2016; El Bouzidi *et al.*, 2011). While the essential oil yield of *Brocchia cinerea* harvested in Algeria was 1.4% (Cheriti *et al.*, 2020)

This difference might be due to several factors, such as the geographical zone of plant species, the degree of plant maturity, climate, soil composition, harvesting time and extraction method. (Elsharkawy *et al.*, 2018; Ramos *et al.*, 2012; 2020).

The results of GC-MS analysis of *Brocchia cinerea* essential oils harvested in the oasis of Tata showed the identification of 17 constituents representing 98.71 % of the total essential oil composition. It's shown in table 1 that the major compounds are Thujone (40.83%), Camphor (16.58%) and Eucalyptol (10.99%). The other most abundant compounds were Santolina triene (9.93 %), 3(10)-Caren-4-ol, Acetoacetic acid ester(6.91%), Camphene (3.00%), Sabinene (2.95%) and α-Pinene (2.01%), The components identified in dried aerial plants were represented by Oxygenated monoterpenes (71.18%), Hydrocarbons (10.31%), Hydrocarbons Monoterpenes (9.38%), Esters (6.91%), Sesquiterpens hydrocarbons (0.68%) and Oxygenated sesquiterpenes (0.25%). Monoterpenes are the major groupe contained in the essential oil of *Brocchia cinerea* while sesquiterpene hydrocarbons were found in negligible quantities and that was the same result revealed by EL bouzidi *et al.* (2011) et chlif *et al*. (2021).

The chemical composition of essential oils from Moroccan *Brocchia cinerea* aerial parts had been examined in previous works. Studies from El Bouzidi *et al.* (2011) and Kasrati *et al* (2015)

described trans-Thujone (41.4%), cis-Verbenylacetate (24.7%), 1,8-cineole (8.2%) and Camphor (5.5%) as the major components of the plants collected in Morocco (Zagora).

Recent study of *Brocchia cinerea* collected in southeast of Morocco (Alnif city) revealed that the major components of dry aerial parts of *Brocchia cinerea* essential oils were Thujone (22.37%), Santolina triene (16.45%), Eucalyptol (12.19%), cis-Chrysanthenyl formate (12.03%), 2-bornanone (11.56%) (chlif *et al.*, 2021). Other studies from Djellouli *et al.* (2015) described (E)-Citral (24.01%), cis-Limonene epoxide (18.26%), Thymol methyl ether (15.04 %), Carvacrol (15.03 %) and Trans-carveol (13.79 %) as the major components of the essential oil of *Brocchia cinerea* collected in Algeria.

Also, another study the chemical composition by GC/MS of the essential oil of *Brocchia cinerea* collected in southeast of Algeria showed in the flowering period the presence of the major compounds: 3-Carène (30.99%), Thujone (21.73%), Santolina triene (18.58%) and Camphor (6.21%). While during the fruiting period the presence of the major compounds: Thujone (28.78%), 3-Carène (15.90%), Eucalyptol (15.13%), Santolina triene (13.38%) and Camphor (7.49%). The last result of this study is in agreement with our results where samples were collected in flowering period (April, May) and dominated by Thujone.

However, the rate of compounds differed substantially from that reported in species collected in Egypt, in which camphor (50%) and trans-Thujone (14.4%) were the main oil compounds (Boukhobza *et al.*, 2020).

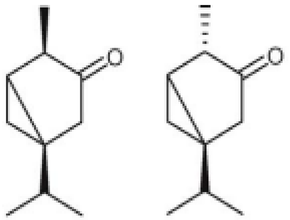
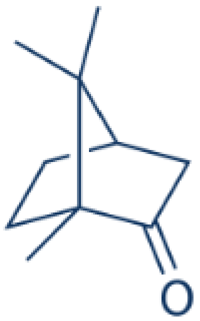
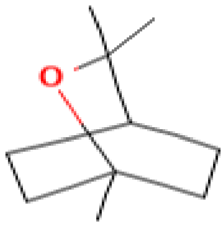
The essential oils of *Brocchia cinerea* collected from the oasis of Tata located in south-east of Morocco are qualitatively and quantitatively different from essential oils collected in other regions. This could be explained by the fact that the compositions of the essential oils are greatly influenced by diverse parameters involving time and season of harvesting of the plants, type of the plant organs, geographical and climatic conditions, physiological age, developmental stage, nutritional status of the plants, post-harvest drying, plant storages, genetic diversity, extraction methods, elemental structure of the sampling area, physicochemical variables related to the respective soils and also stress during the growth or maturity (Raut and Karuppayil, 2014, Tabanca *et al*., 2011; Mohammadhosseini, 2015; Nekoei and Mohammadhosseini, 2016).

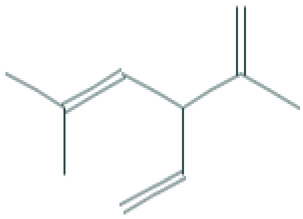
Table 1. Chemical composition of essential oil of *Brocchia cinerea.* RT, Retention time, RI (th), Retention indices reported in the literature, RI (exp): Retention indices calculated.

NO.	**Compounds**	**RT**(mn)	**RI**(th)	**RI**(exp)	**Rate (%)**
1	1,3-diisopropenylcyclobutane	7.33	-	-	0.38
2	3(10)-Caren-4-ol, acetoacetic acid ester	11.84	-	-	6.91
3	Santolinatriene	4.63	903.3	905.9	9.93
4	3-thujene	5.05	925.9	927,2	0.87
5	Alpha–pinene	5.24	934.5	936.9	2.01
6	Camphene	5.74	947.4	953.3	3.00
7	Sabinene	6.23	967.9	970.0	2.95
8	Beta –pinene	6.34	973.1	977.5	0.95
9	1,8-cineol	7.65	1022,4	1021,8	10,99
10	Gamma–terpinene	7.98	1050.3	1054.7	0.47
11	Trans-thujone	9.64	1090.9	1115.1	40.83
12	Camphor	10.58	1125.0	1133.4	16.58
13	Alpha-terpineol	11.07	1175.6	1179.7	0.91
14	Isobornylacetate	12.19	1271.0	1285.0	1.00
15	Beta–farnesene	14.54	1449.3	1455.0	0.68
16	(-)-Spathulenol	17.06	1625.6	1630.5	0.25
Oxygenated monoterpenes [NO, 4, 9, 11- 14]					71.18
Hydrocarbons Monoterpenes [NO, 5- 8; 10]					9.38
Hydrocarbons [No, 1, 3]					10.31
Oxygenated sesquiterpenes [No, 16]					0.25
Sesquiterpens hydrocarbons [No, 15]					0.68
Esters [No, 2]					6.91
Total					98.71

The pharmacological properties of the major compounds Thujone, Eucalyptol, Camphor and Santolinatriene are described in litterature Table 2

Table 2: Pharmacological activities of major compound of *Brocchia cinerea*

Compound	**Reported pharmacological activities**
Thujone  α and β thujone	Toxic, neurocytoxic, insecticidal, antimicrobial, antifungal, cytotoxic and antiviral, antihelmintic, antinociceptive, butyryl- and acetyl-cholinesterase inhibitory activities (Abu-Darwish et al., 2013, Rice and Wilson, 1976, Savelev et al., 2004, Tariq et al., 2009, Sivropoulou *et al.*, 1997; Parliament et Council 2008; Liu *et al.*, 2010, Pelkonen et al. 2013, Hwang et al., 1985, Carta et al. 1996)
Camphor 	Gastrointestinal and central nervous system toxicity, Insecticide, antiseptique, antibacterial, antifungal, antiviral, and cytotoxic, Antimutagenic Anticancer Activity and Cardiovascular Effects (Abivardi, C. 1977, Smith, and Margoli 1954, Kanematsu, and Shibata, 1990, Sivropoulou *et al.*, 1997, Sur *et al.*, 1991; De-Oliveira *et al.* 1997, Belz *et al.* 2000, Parliament et Council 2008, Magiatis *et al.* 2002, Love *et al.* 2004, De Heluani *et al.* 2005, Zhu *et al.* 2005, Kotan *et al.* 2008, Liska *et al.* 2010,)
Eucalyptol 	Antioxydant, anti-inflammatory, insecticidal, antifongal, antimicrobial, cytotoxic and antiviral (Sivropoulou *et al.*, 1997, Klocke *et al.*, 1987; Pattnaik *et al.*, 1997; Santos et Rao, 2000; Lee *et al.*, 2004; Vuuren et Viljoen 2007; Ciftci *et al.*, 2011, Quiroga *et al.*, 2015)
Santolinatriene	Larvicidal (Grace, 2002)

Antibacterial activity

Table 3 shows MICs and MCBs values obtained under closed conditions. The essential oil of *Brocchia cinerea* exhibited antibacterial activity which generally increased in the following order: *Listeria innocua* < *Staphylococcus aureus* <*Pseudomonas aeruginosa*.

In our tests Gram negative *Pseudomonas aeruginosa* bacteria is more sensitive to the essential oil of *Brocchia cinerea* than gram positive bacteria (*Listeria innocua* and *Staphylococcus aureus*).

Brocchia cinerea belongs to the family of *Asteraceae* which is considered as very potent antibacterial agents (Mayekiso *et al.,* 2006, Matasyoh *et al.,* 2007). The antibacterial activity of an essential oil is attributed to its major compounds (Satomayor *et al.,* 2004). Therefore the antibacterial activity observed for *Brocchia cinerea* could be attributed to its major components Thujone, Camphor and Eucalyptol.

According to many previous studies (Nin *et al.*, 1995, Tan*et al.,* 1998; Sivropoulou *et al.,* 1997; Başer *et al.,* 2002; Tsiri *et al.,* 2009, Miladinovićand Miladinović, 2000), Hammer *et al.,* 1999) plants species with Thujone chemotype are recognized to be very powerful antibacterial.

Moreover, In Previous works, the Egyptian sage essential oil, composed mostly of Thujone (41.5%) and of Limonene (14.7%), shows high antibacterial activity against Gram-positive bacteria (Farag *et al.* 1989). Moreover, pure Thujone is a powerful antimicrobial compound (Margaria, 1963; Sivropoulou *et al.*, 1997; Sur *et al.*, 1991)

Also, Eucalyptol is known to possess a strong antibacterial action against *Staphylococcus aureus* (Karlović *et al.,* 2000; Mitić-Ćulafić *et al*., 2009) and against *Pseudomonas aeruginosa* (Nin *et al.,* 1995). Dorman, and Deans (2000) reported that the antibacterial activity of the essential oils

is related to their respective composition, the structural configuration and the functional groups of their constituents and the possible synergistic interaction between components.

In fact, the essential oils of *Salvia macrochlamys* and decorative sage (*S. recognita*), rich in camphor (11% and 42%, respectively), exhibited no antimicrobial activity against *Staphylococcus aureus*,. However, in the same study, camphor tested singularly exhibited no activity against *Staphylococcus aureus.* (Tabanca *et al.*, 2006) but Viljoen*et al*.,(2003) determined, using time-kill studies, that a synergistic antimicrobial effect occurs between 1,8-Cineole and Camphor. The study showed in the case of camphor combined with 1,8-Cineole, a total reduction of colony forming units. It was deduced therefore that Camphor may act in a synergistic manner with other essential oil components possessing antimicrobial activity.

It's probable that the components in lower percentage might be involved in some type of synergism with the other active compounds.

Table 3. MICs and MCBs of tested bacteria. Where MICs is the minimum inhibitory concentrations and MCBs is the minimum bactericidal concentrations

Bacteria	**Gram +/-**	**MIC (µL/mL)**	**MCB (µL/mL)**
S. aureus	+	0.5	4
P. aeruginosa	-	0.6	1.5
L. innocua	+	0.8	3.5

Antioxydant activity

The results of DPPH free radical scavenging activity of the essential oil of *Brocchia cinerea* are presented in table 4.

As shown by the table 4, when compared to BHT and Coviox T_{50}, tested essential oil of *Brocchia cinerea* much less effective than this synthetic antioxidant agent at the examined concentrations.

Oxygenated monoterpenes, are mainly responsible for the antioxidant potential of the plant essential oils which contain them (Aeschbach *et al.*, 1994; Baratta *et al.*, 1998). Monoterpenes, sesquiterpenes and diterpenes have been found to possess notable antioxidant activity in different *in vitro* assays. However, most of these activities have no physiological relevance (Baratta *et al.*, 1998).

The scavenging activity observed for the *Brocchia cinerea* essential oils couldn't be accorded to its major's components, Thujone, Camphor and Eucalyptol

For Thujone, several reports are present on the antioxidant activity of essential oils from various plants, such as *Salvia officinalis* (Walch *et al.*, 2012), *Artemisia herba-alba* (Kadri *et al*., 2011), and *Artemisia absinthium* (Msaada *et al.*, 2015), which contain high amount of Thujone. In all these studies Thujone exhibited low to moderate antioxidant activities of all tested radicals. For Camphor, essential oils dominated by this compound presented weak antioxydant activity. Camphor, 1,8-cineole were previously studied and it was revealed that they displayed weak antioxidant activity (Tepe *et al.*, 2015; Zengin and Baysal, 2014).

Eucalyptol has no effect on reducing DPPH radical (Horvathova *et al.*, 2014).

However, it's difficult to attribute the antioxidant effect of a total essential oil to one or few active compounds. Both minor and major compounds should make a significant contribution to the essential oil's activity (Wang *et al.*, 2008).

In previous works γ-terpinene shows a very high antioxidant activity (Ruberto and Baratta, 2000) and has a comparable activity to that of α-tocopherol **(**Kamal-Eldin and Appelqvist, 1996). The terpenoids α-pinene was determined to be effective DPPH scavengers with showing a strong DPPH free radical scavenging effect (Wang *et al.*, 2019).

Table 4. IC_{50} values (in [mg/mL] ± standard deviation)

Samples	**IC_{50} (mg/mL)**
Essential oil	0.080± 0.014[c]
BHT	0.026±0.002[b]
Cov-iox T_{50}	0.003±0.0004[a]

Indeed and despite of low rate of α-pinene and γ-terpinene (2.09% and 0.47% respectively) of this component in *Brocchia cinerea* essential oil harvested in Morocco could strongly contribute to the scavenging effect observed for this essential oil.

Conclusions:

In this study, we investigated for the first time the chemical composition, antioxydant and antibacterial activities of the essential oil of the medicinal plant *Brocchia cinerea* from Tata (Southeast of Morocco). Chemical analysis of essential oil by GC/MS identified constituents representing 98.71 % of the total essential oil dominated by trans-Thujone (40.83%), Camphor (16.58 %) and Eucalyptol (10.99%). In addition, our results suggest the possibility of using the *Brocchia cinerea* essential oil as a natural preservative for foods as the essential oil of this species have strong antibacterial and antioxidant activities. Further work is necessary to investigate the efficacy, and palatability, of appropriate concentrations of these essential oils in foods.

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