Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(17):240-244
(http://derpharmachemica.com/archive.html)

Phytochemical screening, antioxidant activity and isolation the compounds of *Pulicaria incisa*

Mohamed Samba[a,b], Abderrahmane Hadou[a], Abdi Kaihil[a], Ahmed Ismail Boumediana[a], Mohamed Vadel Deida[a], El Mokhtar Essassi[b] and Mohamed Said Minnih[a,b]*

[a]*Unité de Chimie Moléculaire et Environnement, Université de Sciences, de Technologie et de Médecine, Nouakchott, Mauritanie*
[b]*Laboratoire de Chimie Organique Hétérocyclique, Associé au CNRST, Pôle de compétence Pharchim, Université Mohammed V, Avenue Ibn Batouta, Rabat, Maroc*

ABSTRACT

Medicinal plants in Mauritania are much less known compared to those in the neighboring countries. This study shows that Pulicaria incisa (Lam.) DC is widespread in Mauritania and, in addition, Mauritanians use it for several medicinal purposes, such as relieving colds, flu and fever. Thus phytochemical study of its fruits has allowed us to reveal the presence of alkaloids, flavonoids, tannins, ellagic acid, saponins, sterols, and terpenes. Furthermore it has suggested that the presences of these metabolites are linked to the antioxidant activity of this plant.

Keywords: phytochemical screening, antioxidant activity, *Pulicaria incisa (Lam.) DC*, Mauritania.

INTRODUCTION

Pulicaria incisa (Lam.) DC. subsp. candolleana Gamal Eldin has the synonym-*Pulicaria desertorum DC* (*Asteraceae* family). Nowadays this species is observed in northern Mauritania in different areas, characterised as regs and gravelly soils. It is a therophyte having Afro-Asian distribution [1]. In Mauritania this species is among the mostly used by nomads, mainly as infusion in tea. Also it is advised against colds, flu, palpitations and fever [2]. Extraction of this species permitted us to obtain two products and, in addition to phytochemical screening, biological activity of extracts has been evidenced by antioxydant activity measurement.

MATERIALS AND METHODS

2.1. Plant material

Pulicaria incisa fruits have been collected in March 2013 in eastern Mauritania, Wilaya of Tagant (Tamourt En-naaj extension to Gabbou zone).

2.2. Equipment

Denver TL-series scale and Rota X-Lab S-300 rotary evaporator equipped with a vacuum pump have been use for weighing and evaporation of solvents. For NMR analysis and mass spectrometry we used a Bruker 300 Ultrashield and an Agilent Technologies 6530Accurate -mass Q-TOF LC-MS system, respectively. In addition an optical densitometer has been used to measure optical density.

2.3. Extraction and phytochemical screening

plant material has been extracted with maceration at room temperature. Phytochemical tests were performed according to standard protocols characterization. Alkaloids have been detected with Dragendorrff reagent [3], gallic

tannins by addition of iron trichloride [4], saponins, ellagic acid, flavonoids, proanthocyanidins and quinones have been detected according to the experimental procedures described by [5] [6] and [6]; sterols and terpenoids have been evidenced by Lieberman-Burchard and Salkowski reactions [7].

2.4. Antioxidant activity

Antioxidant activity has been determined spectrophotometrically according to the Brand- WILLIAMS et al method [7], using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as free radical. A solution of 0.1 mM DPPH and Trolox standard solutions were prepared at different concentrations (1 / 1.5 / 2/3/4 mM) in methanol. 0.1 ml of Trolox / MeOH solutions were mixed with 10 ml of the solution of DPPH / MeOH and then the absorbance of the solutions was measured after 30 minutes at 517 nm. 0.1 ml of the extract was mixed with 10 ml of the DPPH/MeOH solution and thenafter the absorbance of this solution was measured after 30 minutes at 517 nm. Antioxidant activity is given in mmol Trolox equivalent (TEAC) / 100 g dry matter. The antiradical activity is expressed as inhibition percentage (% I) of free radicals using the following formula:

I% = [1- (Sample Abs - Abs negative control)] x 100
Where: I%: percentage of the anti-radical activity (AAR %)
Abs Sample: Sample Absorbance;
Abs negative control: Absorbance of negative control

2.5. Extraction and isolation of the constituents

An amount of the air port (400g) of the plant was dried, ground and finely macerated for 24 hours in a methanol-water mixture (80:20 v/v).The last operation has been repeated three times. After filtration and concentration at each time, the obtained aqueous phase has been extracted several times with cyclohexane. Cyclohexane phases have been combined and dried over sodium sulphate and then after evaporated to dryness. A mass of 5.4 g of the cyclohexane extract was obtained. Aqueous phase is then taken up with chloroform and ethyl acetate using the same protocol, giving 7 g and 8.4 g of the chloroform extract and ethyl acetate, respectively. Finally, aqueous phase is depleted with butanol after drying with Na_2SO_4 followed by filtration and concentration, thus obtaining 24 g from butanol extract. Study of ethyl acetate extract

Ethyl acetate extract (8.4 g) underwent a first column chromatography fractionation on silicausing a Cyclo / EtOAc gradient to afford seven fractions (F1 to F7). Fraction F2 (12mg) was chromatographed on a preparative plate with cyclohexane as eluting system / ethyl acétae: 6/4 to give compound A (4mg). The fraction F5 itself has undergone a fractionation by adsorption chromatography on silica using a gradient of cyclohexane / ethyl acetate thereby obtaining five sub-fractions, the subfraction F5.3 contained a major product which precipitated in solution ($CHCl_3$ / ethyl acetate 85: 15) forming a white powder. This precipitate was washed with chloroform to give compound B.

Compound C1

Compound C1: 2-hydroxy-4, 6-dimethoxyacetophenone
The ^{1}H NMR spectrum (Table 3) showed two meta aromatic hydrogens at δ 6.08 and 5.94 (d, J = 2.2 Hz). In addition, acetophenone group has been inferred from the methyl group at δ2.63ppmand confirmed by ^{13}C NMR spectrum (Table 3) at δ32.97 ppm and acetophenone carbonyl at δ203.18ppm. The additional substituents of the aromatic ring have been defined as one quelated hydroxyl at δ14.06 and two methoxyls observed at δ3.87ppm (δ55.57ppm) and δ3.84ppm (δ55.57ppm). ^{13}C NMR spectrum (4.9 T) showed signals corresponding to four quaternary aromatic carbon atoms (δ105.99ppm; δ167.59ppm; δ166.07ppm; and δ162.89ppm); and two aromatic methynes (δ90.74ppm and δ93.44ppm).

Compound C2: dihydroflavonol**: m.p 225-227** °C.
Compound (C2): crystalneedles, ESI$^+$ m / z 289 [M + H]$^+$, resonances assignable to two aromatic protons meta-coupled at δh 5.94ppm (1H, d, J = 1.8 Hz, 6-H) and δh5.90 ppm(1H, d, J = 1, 8 Hz, H-8), four A2X2-type aromatic ring protons at δH 7.37 ppm(2H, d, J = 8.5 Hz, H-2', 6') and δh6.85 (2H, d, J = 8.5 Hz, H-3', 5'), two coupled oxymethine protons at δH 5.06 ppm (1H, d, J = 11 ,3 Hz, H-2) and δh4.54ppm (1H, d, J = 11,3 Hz,H-3). ^{13}C NMR spectrum (Table 1) showed a typical downfield resonance at δC 197.10 ppm, characteristic of a C-4 carbonyl resonance of a flavanonol skeleton (Sakushima et al., 2002).

RESULTS AND DISCUSSION

3.1. Extraction and phytochemical screening

Table 2 which summerizes phytochemical screening analysis of *Pulicaria incisa* extracts shows that aerial part contains numerous phytochemical families, namely alkaloids, terpenoids, sterols, saponins, flavonoids, ellagic and

tanninacid. In the contrary this study shows that chemical classes of quinones and proanthocyanidins are not present (Table 1).

Table 1: Different groups of compounds highlighted in Pulicaria incised

phytochemical compounds	Reaction / reagents	Observations
Tannins	SR ,HCl	+
Saponins	MT	+
Polyphenols	FeCl3 à 2%	+
Proanthocyanidols	HCl	-
Ellagic acid	Acetic Acid	+
Flavonoids	RC	+
Sterols and terpenes	LR	+
Quinones	BGR	-
Alcaloids	DR , BR	+

RS= Reagent Stiasny; MT= Mousse test; RC= Reaction to Cyanidin; LR= Liebermann reaction; BGR = Borntraeger reagent; DR= Dragendorff reagent; BR= Burch

3.2. Antioxidant activity measurement

Evaluation of the antioxidant activity by the DPPH test, expressed as percentage of the anti-radical activity (Figure 1), revealed an strong antioxidant power for all extracts.

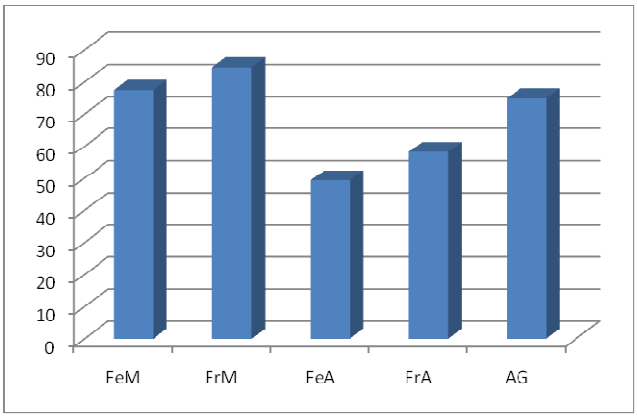


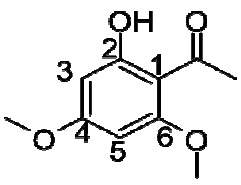
Figure 1: Percentage inhibiting DPPH

GEF = methanol sheet; Frm = Flower Méthnolique; FEA = Aqueous Leaf; FrA = Aqueous Flower; AG = Gallic Acid

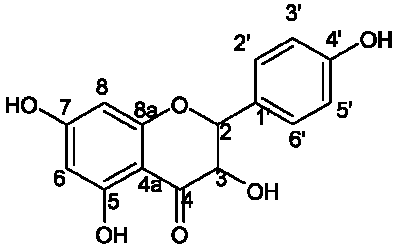
Methanol extract has the most powerful antioxidant effect (84,64% and 77.57% for flowers and leaves, respectively), whereas aqueous extracts present a lower activity in comparison to the methanol ones (58.23% and 49.34% for flowers and leaves respectively). Also these results show that the activity of flower extracts is stronger than that of leaves; which may be explained by the richness of polyphenols presence in flowers. Gallic acid; which shows a powerful antioxidant activity, 75.5%, has been used as reference, in order to compare extracts activities.

3.2. Separation and purification of *Pulicaria incisa* extracts

A hydroalcoholic extract of the dried *Pulicaraia incisa* seeds has been carried out (see § 3.1). Subjection of the ethyl acetate soluble fraction (8.4 g) to column chromatography has given seven fractions (F1 to F7). Thereafter fractions F2 (12 mg) and F5 (70mg) were chromatographed to give compound 1 (4mg) and compound 2 (54mg), respectively.



Compound 1



Compound 2

Compound 1

Integration of the various components of proton NMR signals shows that the structure has twelve protons that resonate at different chemical shifts. HMQC-NMR spectrum confirms this finding and indicates the presence of two aromatic protons, two methoxy groups (-OCH3) and a methyl group (-CH3). Furthermore, COSY spectrum shows the correlations between the two aromatic protons and also between the protons of two méthoxy groups (chemical

shifts 3.87 ppm) with aromatic protons, while the protons of other methoxy group present a small spot correlation with the protons of the methyl group.

In the following discussion of HMBC spectrum, we will adopt the numbering indicated on the structure above (compound 1). The protons of the methyl group correlate with C7-carbon of the carbonyl function at δ = 203.18 ppm and the quaternary carbon, C1 (δ = 105.99 ppm). Protons of methoxy group in position 4 has a correlation with the C2 carbon (δ=162.89 ppm), whereas protons attached to methoxy group in position 6 correlates with C4 carbon (δ =166.07ppm).

Aromatic proton resonating at 5.94 ppm carried by the carbon in position C3 (δ = 90.74ppm) has a correlation with the carbon C4 and C1(δ = 105.99 ppm), and a small correlation spot with the C5 carbon (δ = 93.44 ppm). In addition the other aromatic proton carried by C5 (δ = 93.44 ppm) correlates with the carbons C1 (δ = 105.99 ppm) and C3 (δ = 90.74 ppm). Additionally 1H NMR spectrum showed two aromatic hydrogens in position meta to δ 6.06ppm and 5.92ppm (δ, J = 2.2 Hz). Therewith, the acetophenone group was deducted from the methyl group at δ = 2.63 ppm and confirmed by ^{13}C NMR spectrum at δ = 32.97ppm and acetophenone carbonyl at δ = 203.18ppm.Supplementary substituents on the aromatic ring have been defined as a hydroxyl group at δ 14.06 ppm and two methoxyls observed at δ 3.87 ppm (δ 55.57ppm) and δ 3.84ppm (δ 55.57ppm).

The NMR spectrum showed ^{13}C signals corresponding to four quaternary aromatic carbons (δ 105.99 ppm; 167.59ppm; 166.07ppm; and 162.89ppm) and two aromatic CH (δ 90.74ppm and 93.44ppm).

Table 2: Comparison 1 and Literature

Position	δH/ppm	δC/ppm	δH/ppm literature	δC/ppm literature
M e(CO)Ar	2,63	32,97	2.61 [3H, s,]	32.9
6-OMe	3,84	55,57	3.81 (3H, s,),	55.6
4-OMe	3,87	55,57	3.85 (3H, s,),	55.6
1		105,99		106
2		162,89		162 ,9
3	5,94 (lH, d,J=2.2 Hz,),	90,74	5.92 (lH, d, J=2.4 Hz,),	90.7
4		166,07		166 ,1 1
5	6,08 (lH, d, J=2.2Hz,),	93,44	6.06 (lH, d, J=2.4 Hz,),	93.5
6		167,59		167,6
2-OH	14,06		14.04 (1 H, S,);	
CO		203,18		203,2

Table 3: NMR assignment of Compound 1

Position	δH	δC	HMBC	COSY
M e(CO)Ar	2,63	32,97	C7, C1	
6-OMe	3,84	55,57	C4	
4-OMe	3,87	55,57	C2	
1		105,99		
2		162,89		
3	5,94 (lH, d,J=2.2 Hz,),	90,74	C4,C1,C5	H5
4		166,07		
5	6,08 (lH, d, J=2.2Hz,),	93,44	C1,C3	H3
6		167,59		
2-OH	14,06		C1, C4,C5	
CO		203,18		

Structure determination of Compound 2

Compound 2 is in the form of a white powder soluble in methanol. It reacts with the Neu reagent displaying a blue fluorescence under UV at 365 nm, suggestion flavonoid-like structure. Mass spectrum in the positive mode electrospray (ESI+) indicates a quasi molecular ion at m/z 289 $[M+H]^+$ suggesting a molecular mass of 288. Compound 5 has the molecular formula $C_{15}H_{12}O_6$. H-NMR spectrum of compound C2, recorded at 300 MHz in deuterated methanol (Table 2) shows three characteristic group signals of flavonoid rings A, B and C. Integration of the proton spectrum signals indicates that the structure has 12 protons and HMQC-NMR spectrum confirms this finding.^{13}C NMR spectrum of this compound has twelve distinct signals corresponding to twelve carbons of a flavonoid. Between these twelve carbons, we distinguished seven quaternary ones of which a carbonyl detected at **δ**C 197.10 ppm and the carbons 2 and 3, characteristic of a dihydroflavonols (**δ**C 72.21ppm and 83.55ppm, respectively), in addition to four aromatic CH.

Analysis of HMBC spectrum indicates that H-6 proton correlates with carbons C-4a (100.63 ppm), C-5 (163, 90 ppm), C-7 (167, 79ppm) and C-8 (95,09 ppm).

Table 4: RMN of Compound 2

Position	δC/ppm	δH/ppm	HMBC	COSY
2	83,55	5,06(1H,d,J=11 ,3Hz)	C-1', C-4, C-3, C-2' ,C-6'	H-3, H-2',H-6'
3	72,21	4,54(1H,d,J=11,3Hz)	C-4, C-1' , C-2	H-2
4	197,10			
4a	100 ,42			
5	163,89			
6	95,98	5,94(1H,d, J=1 ,8Hz)	C-7, C-5, C-4a, C-8	
7	167,29			
8	94,85	5,90 (1H,d, J= 1,9H)	C-7, C-6, C-4a, C-8a	
8a	163,13			
1'	127,85			
2' et 6'	128,97	7,37(2H, d, J=8,5 Hz)	C-2,C-3', C-5',C-4	H-3' , H-5'
3' et 5'	114 ,71	6,85 (2H, d, J=8,5Hz)	C-2', C-6', C-1', C-4'	H-2', H-6'
4'	157,81			

Table 5: Comparison of compound 1with literature

Position	δC/ppm	δH/ppm	δC/ppm literature	δH/ppm literature
2	83,55	5,06(1H,d,J=11 ,3Hz)	83,5	5,27(1H,d, J=11,4)
3	72,21	4,54(1H,d,J=11,3Hz)	77,7	4,95(1H,d,J=11,4)
4	197,10		196,6	
4a	100 ,42		102,4	
5	163,89		165,6	
6	95,89	5,94(1H,d, J=1 ,8Hz)	97,4	5,91(1H,d, J=2Hz)
7	167,29		169,1	5,89 (1H, d , J= 2Hz)
8	94,85	5,90 (1H,d, J= 1,9H)	96,4	
8a	163,13		164,2	
1'	127,85		128,6	
2' et 6'	128,97	7,37(2H, d, J=8,5 Hz)	130,6	7 ,31 (2H,d, J=8,6)
3' et 5'	114,71	6,85 (2H, d, J=8,5Hz)	116	6,78 (2H,d, J= 8,6)
4'	157,81		159	

CONCLUSION

The phytochemical study has shown the presence of several secondary metabolites, which present a potential economic and medicinal interest. On the other hand, the study of the antioxidant activity has showed the presence of a variable activity, for both the methanolic and aqueous, fruits and leaves extracts of *Pulicaria Incisa*. Finally, analysis of polyphenols from leaves and fruit of this plant Has helped to highlight the presence of two known polyphenolic compounds, 2-hydroxy-4, 6-dimethoxyacetophenone and dihydroflavonol

REFERENCES

[1]AI Boumediana, Thèse de Doctorat d'Etat, Université Cheikh Anta Diop (Dakar, **2013**).
[2]Rouane, Mémoire de Magister, Université des Sciences et de la Technologie Houari Boumediène (**2012**).
[3]GE Trease, WC Evans, A Text Book of Pharmacognosy. ELSB/Bailliere Tindal,(Oxford, **1987**).
[4]EA Sofowora, Medicinal Plants and Traditional Medecine in Africa. Wiley, Chichester, **1982**.
[5]JB Harborne, Chapman, Itall. Phytochemical methods. New-york, **1973**.
[6]WB Williams, ME Cuvelier, C Berset, Use of a free-radical method to evaluate antioxidant activity, Lebensmittel – Wissenschaft und Technologie, **1995**, 28, 25-30.
[7]C Richard, Cambie, R Allick, Lal, S Peter, Rutledge, D Paul, Woodgate *Phytochemistry*, **1991**, 30, 287-292.
[8]KW Hiroyoshi, L Michihiko, K Yinghua, W Kazuo, Koike *Phytochemistry*, **2010**, 71, 1925-1929.