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Phytochemical Investigation of the Bioactive Extract from *Launeae arbrescens*

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Abstract: In this study, launea arborescens (Asteraceae) was selected for phytochemical investigation. The important activity of the methanol extract detected in the biological and chemical screening performed previously and the lack of studies concerning the genus prompted this choice. The aerial parts methanol extract of launea arborescens (Asteraceae) displayed several activities: amongst these, the antifungal activities against *Candida albicans* and *Sacharomyce cereviveae* and the antibacterial activity against *E. coli*, *S. aureus*, *P. aeriginisa* and *Klebseila entrecocus*. The extracts presenting the most interesting activities are then selected for activity-guided phytochemical investigation, in order to identify the active compounds. Moreover, the isolation and characterization of further molecules presenting original chemical structures and a potential therapeutic interest is performed.

Key words: *Launea arborescens*, flavonoid, bioactive extract, chemical screening

INTRODUCTION

In the strategy of research of new bioactive substances of plant, we followed a method of bio guidance based on the isolation of the constituent of the most active extracts of this plant for every microbial stump (Belboukhari and Cheriti, 2006). The survey of the biologic activity by the different antifungal and antibacterial tests watch that the methanol and aqueous extract have some important antimicrobial activities; one starts our survey with a chemical screening on the different classes of natural products (Rogers *et al*., 1996).

Our work consists has determine in a first stage the number of constituent in methanol extract by the fractionation method of the crude extracts in three solvent has different polarities. In the second stage, one is interested in to isolate the constituents of the fraction of ethyl acetate by the classic chromatography methods (Cheriti *et al*., 2004).

MATERIALS AND METHODS

General experimental procedure: The IR spectra were obtained with a Perkin Elmer 1710 spectrophotometer. The NMR spectra taken on a Bruker GP× 250 (P^1H, 250 MHz; $P^{13}C$, 125 MHz), EIMS spectra were obtained on a VG trio-2 spectrophotometer. The TLC was carried out on silica gel 60 FB_{254}. Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh) and Sephdex LH-20.

Plant materials: The whole plants of *Launeae arborescens* (Asteraceae) was collected in March 2000 from Bechar (hammada oued saoura) south of Algeria (Quezel and Santa, 1963; Ozenda, 1983). A voucher specimen has deposited in the Phythochemistry herbarium of Phytochemistry and organic synthesis Laboratory of University center of Bechar under to accession number CA99/25 (Belboukhari *et al*., 2005).

Extraction and isolation: The dried aerial part plants (30 g) of *Launeae arborescens* (oum lbina) extracted with 80% MeOH (300 mL) in an soxlet apparatus for 1 h after filtration, this residue was evaporated in vacuum and according to the operative fashions of chemical screening one determines the present natural substances in bioactive extract (Bruneton, 1999). This extract was then suspended in distilled water and partioned sequentially with n-Hexane, ether, ethyl acetate and n-butanol. The analysis of extracts is achieved by the TLC method using an eluent, acetone: toluene: formic acid (6: 8:1) (Rosset *et al*., 1991). The ethyl acetate fraction (0.56 g) was subjected to silica gel column chromatography (20 g) using a mobile phase: ethyle acetate: methanol with the report in following volume: (30: 4.5) (Cheriti *et al*., 2005). The recovered fractions are analyzed by TLC of which the used eluent is the mixture, Benzene: acetone with the report of volume (3:7) (Hostettman *et al*., 1968).

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RESULTS AND DISCUSSION

Chemical screening: According to the gotten results one notes that methanolic extract of *Launeae arborescens* contains the following natural substances: tannins exist in considerable quantity, terpenes and cardinolids present a weak quantity and a percentage average for saponins and the free flavonoids. One notices the absence of stérols and alkaloids (Belboukhari *et al.*, 2002). The analysis by TLC shows that there are 4 products in the ether diethylique extract and 5 products in the ethyl acetate and the Butanol extracts of this plant (Table 1).

The TLC analysis shows four products separated of the ethyl acetate extract: OLMAETC01, OLMAETC03, OLMAETC06, OLMAETC07, after the drying the determination of structures is achieved by spectroscopic methods: UV, IR, NMR. For miscellanies of two products are recovered to do a second column of chromatographie. The analysis by TLC reveals the separation of 6 products of the ethyl acetate extract in the second column of chromatographie.

OLMAETCC01: 3-Acetyl-5-methoxy-7,3', 4'-triHydroxy-8-O-glucoside-flavan-3-ol (1): Yelow powder, mp = 145° C, UV max (MeOH): 231, 281, IR (KBr, cm^{-1}): 3453, 2951, 2923, 2847, 1738, 1629, 1470, 1156, 1383, 755.

^{1}H NMR ($CDCl_3$): 7.06(d,1.5, H-2'), 6.80(dd, 8.0, 1.5, H-6'), 6.16(s,H-6), 5.38(s,H-3), 5.01(s,H-2), 4.68(d, 7.5, H-1"), 3.79(s,CH_3), 3.76(m,H-6"), 3.39-3.48 (H-2", H-3", H-4"), 3.24 (m, H-5"), 2.96(dd, 17.5, 4.5, H-4a), 2.84 (dd, 17.5, 2.0, H-4b), 1.87(s,CH_3).

^{13}C NMR ($CDCl_3$): 78.38(C-2), 69.33(C-3), 26.62 (C-4), 152.55 (C-5), 93.79 (C-6), 153.33(C-7), 128.68 (C-8), 149.43(C-9),101.16 (C-10), 130.92(C-1'), 115.01 (C-2'), 145.98 (C-3'), 146.01 (C-4'), 115.98 (C-5'), 119.04(C-6'), 106.66(C-1"), 75.75(C-2"), 77.66(C-3"), 71.13(C-4"), 78.00(C-5"), 62.51(C-6"), 172.01(CO), 56.83 ($OCHB_3$), 20.77(CHB_3).

OLMAETCC02: 5,7,4'-trihydroxy, 3'-methoxy flavone (2): The MS specta of this compound watch a pseudo-molecular ion to m/z 301 [M+H]+ and one fragment to m/z 286 [corresponding M-CH3+H]+ to the loss of a methyl. These data and the UVS specter indicate that is probably an aglycone of type flavone with one group méthoxyles and three hydroxyl groups, but the MS2 specter doesn't permit to observe the A+S ions and resulting B+ of the rupture of the cycle C.

UV max (MeOH): 250, 269, 345. The post - column addition of the sodium acetate misleads a displacement bathochrome of the II strip (18 nm) in the UV specta of 2, what shows the presence of a group hydroxyle in, position 7. The apparition of a second maximum in the strip I to 379 nm, observed with the reactive $AlCl_3$/H+, is revealing of a displacement induced bathochrome by a 5 OH. In relation to this specter, the addition of the solution $AlCl_3$ doesn't show meaningful displacement of maximas of the I strip what indicates the absence of two groups free ortho-hydroxyleses on the B cycle. The UV specter gotten, with the NaOAc permits to observe a group hydroxyle in 4 'S on the other hand by displacement bathochrome of 62 nm of the I. strip The presence of a épaulement in the II strip of the UV specter in the éluant indicates the presence of one otherwise system 3',4'-dioxygéné, what permits to finally localize group méthoxyle some, S. The compound is therefore the derivative 3' OH methyl of the lutéoline, the chrysoériol.

Table 1: The analysis by TLC of fraction

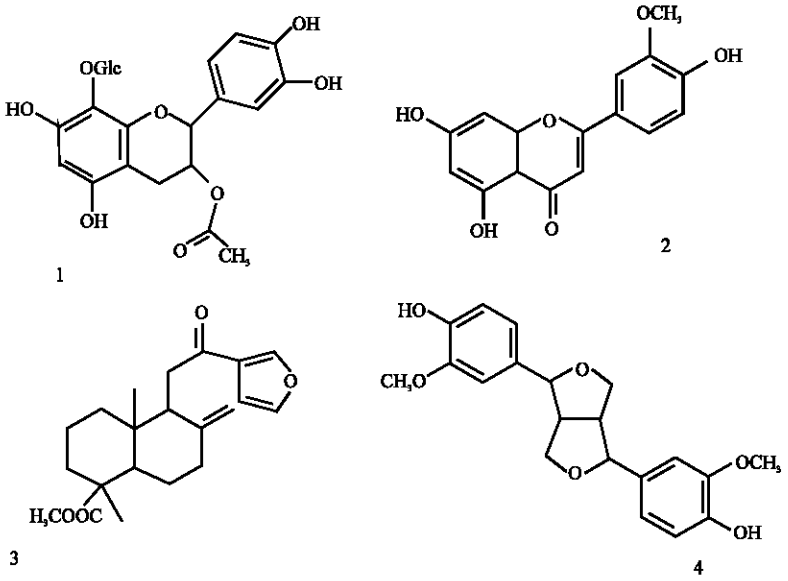
Extract	yield	Color of extract	Rf	color λ (UV) 365 nm
			0.07	
Ether	2.2	green sallow	0.19	Mauve
			0.30	
			0.49	
			0.07	Mauve
			0.16	
Ethyle	3.5	Yellow	0.28	
acetate			0.49	
			0.94	
			0.07	
			0.21	
Butanol	2.1	brown yellow	0.33	
			0.46	
			0.51	Mauve

OLMAETC03: $C_{21}H_{28}O_4$: Me-15,16-époxy-12-oxo-8 (17),13 (16), 14ent-labdatrién-19-oate (3): an amorphous white gunpowder, UV (MeOH): MP^+ = 344 [39], ^{1}H NMR: 1.21 (H-1a, m), 1.65(H-1b, m), 1.52 (H-2a, m), 1.86(H-2b, M), 1.08(H-3a,m), 2.19 (H-3b, m), 1.47 (H-5, dd, 2.9,12.7), 1.78 (H-6a,m), 2.02 (H-6b,m), 2.09 (H-7a,m), 2.41(H-7,m), 2.66 (H-9,d,9.8), 2.76 (H-11a, dd, 3.4, 16.6), 2.96 (H-11b, dd,9.8, 16.6), 6.77 (H-14,d,9.8), 7.43 (H-15, t, 1.5), 8.08 (H-16, s), 4.37 (H-17a, s), 4.74 (H-17b, d, 1.0), 1.21(H-18, s), 0.60(H-20, s), 3.63(OCH_3, s).

^{13}C NMR: 39.3 (C-1), 19.8 (C-2), 38.1(C-3), 44.3 (C-4), 55.9 (C-5), 25.7 (C-6), 37.9 (C-7), 148.7 (C-8), 50.3 (C-9), 39.5 (C-10), 36.4 (C-11), 194.2 (C-12), 128.0 (C-13), 108.7 (C-14), 144.1 (C-15), 156.6 (C-16), 106.4 (C-17), 28.7 (C-18), 177.6 (C-19), 13.0 (C-20), 51.2 (OMe)

OLMAETC01: 4,4'-Dihydroxy-3,3'-diméthoxy-7,9':7',9-diépoxylignane (4): EI-MS (70 eV), m/z (rel. int.): 358 [M].+, UV (MeCNaq): 203, 231, 281

^{13}C NMR (125 MHz, CD3OD) 133.8 (C-1, C-1'), 111.0 (C-2, C-2'), 149.1 (C-3, C-3'), 147.4 (C-4, C-4'), 116.1 (C-5, C-5'), 120.1 (C-6, C-6'), 87.5 (C-7, C-7'), 55.4 (C-8, C-8'), 72.6 (C-9, C-9'), 56.5 (3-CH_3, 3'-CH_3).



^{1}HNMR: 6.95 (H-2,2', d, 2.0 Hz), 6.77 (H-5,5', d, 8.3 Hz), 6.81 (H-6, 6', dd, 2.0, 8.3 Hz), 4.71 (H-7, 7', d, 4.4 Hz), 3.14 (H-8, 8', m), 3.84 (H-9a, dd, 3.4, 8.8 Hz), 4.23 (H-9b, dd, 6.8, 9.3 Hz), 3.85 (3-OMe, s)

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