Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(2):17-21
(http://derpharmachemica.com/archive.html)

Triterpenoid and falvonoid from aerial part of *Launaea arborescens*

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ABSTRACT

Launaea arboresens (local name "Oum Lbina") is an endemic herbaceous medicinal plant mainly distributed in the southwest of Algeria and southeast of Morocco. Chemical investigation of Launaea arborescens resulted in the isolation of a series of triterpenes , flavonoids and tannin from both the aerial parts . Seven compounds have been chemically characterized and identified by using spectroscopic methods as : ursane-12-ene-3,6,16,21-tetraol-3-O-β-glucopyranoside , β—sitostérol, olean-12-ene-3b,16b-diol , 7-O-(α-ramnopyranosyl-(1-6)-β-glycopyranosyl)- 6-Geranyl-5,2',4'-trihydroxy-flavanone , 5,7,4' trihydroxy-3-(3 ,6-dimethyl-2,5-heptadienyl) flavones , 2'',3'',6,8 – Tetrahydroxy-3',4',5',5-tetramethoxy-10,4- cyclolignan and3,4-Di-O-galloylglucopyranoside.

Keywords: *Launaea arborescens*, Asteracea, triterpenoid, flavone, flavanone, tannin, sitocterol,

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INTRODUCTION

The species *Launaea arborescens* belongs to the one of the vastest family of the vegetable kingdom, *Asteraceae*. In the flora of Algeria , five of the nine current species of *Launaea* are endemic of North Africa and include *Launaea arborescens* (Batt.) Murb, of synonym *Zollikoferia spinosa* ,[1,2]. Despite to the pharmacological interest in this plant (local name ''Oum Lbina") commonly used in the North African popular medicine against diarrhoea and abdominal spasms. a very few chemical studies on *L. arborescens* have been so far reported. The methanol extract of *Launaea arborescens* showed antifungal activity against *Candida albicans* and *Saccharomyces cerevisae* and antibacterial activity against gram +*Staphylococcus aureus* and gram – *Escherichia coli*, Pseudomona. *aeruginosa* and *Klebsiella entrecocus [3]* . To the best of our knowledge, only flavonoid, phenolic, essential oil and sesquiterpenes and triterpenes constituents have been described in the literature[4-7].

Our work consists has determine in a first stage the number of constituent in water/ acetone extract by the fractionation method of the crud extracts in three solvent has different polarities. In the second stage, one is interested in to isolate the constituents of the fraction of Butanol and ethyl acetate by the classic chromatography methods [8].

MATERIALS AND METHODS

General experimental procedure

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

Plant materials

The whole plants of *L. arborescens* were collected in Bechar (hammada oued saoura) [1,9] south of Algeria during flowering in March 2000. A voucher specimen has deposited in the phytochemistry herbarium of phytochemistry and organic synthesis Laboratory of University center of Bechar under to accession number CA99/25 [10] . The aerial parts of the plants were separated from the roots and both were allowed to dry before the extraction.

Extraction and isolation

The dried aerial part plants of *Launaea arborescens* extracted with water/acetone in An soxlet apparatus for 1h 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. This extract was the suspended in distilled water and portioned 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 acetone: formic acid (6:8:1) [11] . The ethyl acetate and n-butanol fractions were subjected to silica gel column chromathography (20 g) using a mobile phase : (ethyl acetate : methanol) with the report in following volume: (30:4.5) [12] . The recovered fractions are analyzed by TLC of which the used eluent is the mixture (benzene: acetone) with the report of volume (3:7) [13].

RESULTS AND DISCUSSION

The TLC analysis shows seven products separated of the butanol fraction : and one product separated from the ethyl acetate fraction, after the drying the determination of structures is achieved by using spectroscopic methods.

The negative HR-ESI-MS of compound 1 gave pseudomolecular ion peak at m/z 635.41[M-H]+, in agreement with a molecular formula of $C_{36}H_{60}O_9$. Compound 1 displayed 36 carbon resonances (16.8,17.4, 18.2, 18.7,18.8,22.9 and 25.0) and two sp2-hybridized carbon signals(126.0 and 138.6), signals for four hydroxymethine groups (67.0 , 67.3, 70.5 and 89.3) could be observed. Spectra NMR shows typical spectral characteristics of the triterpene and according to this interpretation we propose the structure partial of triterpenic saponin , compound 1 established as being the ursane-12-éne-3,6,16,21-tetraol -3-O-β-glucopyranoside (4 .1). These results in conformity with those ofLALITA et al research work, wich this compound was isolated from sheets and stems of *Silphium radula* (asteraceae) [14].

Data of ^{1}H NMR and IR characterizing a phytosterol carrying a grouping hydroxyl on C-3 carbon and a double connection in C5-C6 [15] . The whole of spectral data of compound 2 compared with those of the literature is in agreement with the structure of the β-sitostérol [16-18] . This compound was isolated from the sheets of *Saxifraga* plant will *stolonifera* (L) Meeb (*Saxifragaceae*) [19] . Thus of sheets of *Millettia versicolor* (Fabaceae) [20].

The structure partial proposed of the made up of compound 3 isolated from ethyl acetate fraction is Triterpene maniladiol and the known: olean-12-ene-3b, 16b-diol. This compound was already isolated starting from the flowers from *Chrysanthemum morifolium* in the *asteraceae* family [21] . The signal byte 1.30-1.61 ppm (m) with intensity (35H) show a series of very dense peaks . This supposes the presence of a high number of primary carbons, secondary and tertiary (CH_3, CH_2 and CH) in the carbonaceous chain of the molecule. This explains the complexity of the signals observed in this zone.

On the other side the fraction of the butanol extract was characterised by two flavonoid metabolites , compound 4 is a geranyl glucosidic flavanone, which is thus the 7-O-(α-ramnopyranosyl-(1-6)-β-glycopyranosyl)- 6-Geranyl-5,2',4'-trihydroxy-flavanone . This compound had never been quoted so far in the literature according to research bibliography. It is thus about a new natural product.

The spectrum ^{1}H NMR of compound 5, directs towards the structure partial of a prenyl flavone, which is thus the 5,7,4' trihydroxy-3- (3, 6-dimethyl-2,5-heptadienyl) flavone.

The analysis of our spectroscopic results and in particular the chemical shifts are in perfect agreement with the structure partial of a ellagitannin, which is thus the 2", 3", 6,8 - Tetrahydroxy-3', 4', 5', 5-tetramethoxy-10,4-cyclolignan .The whole of these spectral data of compound 6 compared with those of literature [22,23].

The analysis of our spectroscopic results of compound 7 is in perfect agreement with the structure: 3,4-Di-O-galloylglucopyranoside.These results are confirmed in addition by the comparisons with the data of the research works of HUSSEIN et al , FENG et al and KLAUS et al [22,24,25].

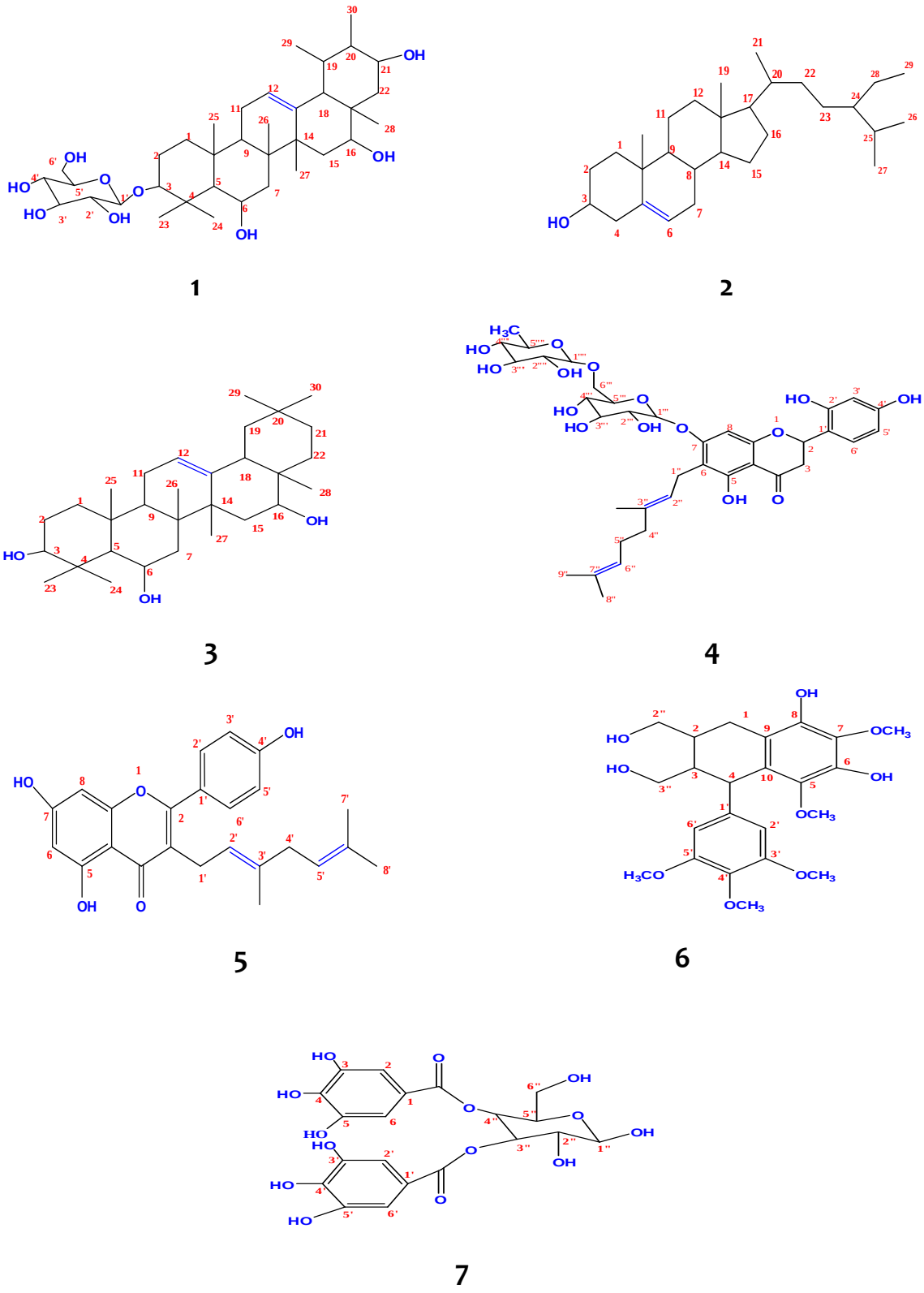

Fig.1 : structure of compound 1-7 (1: *ursane-12-ene-3,6,16,21-tetraol-3-O-β-glucopyranoside , 2:* β—sitostérol, olean-12-ene-3b,16b-diol, 7-O-(α-ramnopyranosyl-(1-6)-β-glycopyranosyl)- 6-Geranyl-5,2',4'-trihydroxy-flavanone , 5,7,4' trihydroxy-3-(3 ,6-dimethyl-2,5-heptadienyl) flavones , 2'',3'',6,8 –Tetrahydroxy-3',4',5',5-tetramethoxy-10,4- cyclolignan and*3,4-Di-O-galloylglucopyranoside*

Compound 1 : *ursane-12-ene-3,6,16,21-tetraol-3-O-β-glucopyranoside.isolated from butanol fraction*
ESI-MS mz : 635.41[M-H], 473[M-H-162] ^{1}H NMR ($CDCl_3$) δ 5.37 (d, 4.9,H-12), 5.32 (d,7.5,H-1'), 3.83 – 3.65 (m ,H-2' ,H-3',H-4', H-5' ,H-6'), 3.70 (d, 6.0, H-3), 3.57-3.62 (m,H-6,H-16,H-21), 2.30-2.39 (m ,H-11 ,H-18) ,2.16 – 2.10 (m ,H-2), 1.83 – 1.71 (m,H-1), 1.60-1.64 (m, H-22,H-19), 1.42 (d, 7.3 ,H-15), 1.42 (d ,7.3,H-15), 1.33 (m ,H-20,H-7),1.12 (s ,H-25,H-26) 1.01 – 0.95 (m,H-5), 0.92
(s ,H-27), 0.90 (s,H-23) 0.89 (s ,H-28), 0.85 (s ,H-24) 0.75 (d, 5.2,H-30), 0.69 (d, 6.7,H-29).

Compound 2 : β--sitostérol White powder isolated from butanol fraction ; mp:135°C, IR (KBr, cm^{-1}) 3415 ,2923,1612,1389, 1071, 797 ; ^{1}H NMR ($CDCl_3$) 5.35 (d,5,H-6), 4.14 – 4.10 (m,H-3),2.04 – 1.99 (m,H-7,H-4), 1.70 – 1.54 (m,H-1,H-7,H-12,H-15,H-16), 1.38-1.47 (m,H-8,H-11,H-17,H-20,H-22,H-23,H-25), 1.24 (dd, J = 8.0, 6.1 Hz), 1.00 (s ,H-19), 0.95 (d,6.5,H-21), 0.91-0.93(m,H-9,H-14,H-24) (s), 0.86 (d, 6.7,H-26 ;H-27),0.79-0.84 (m,H-29), 0.68 (s,H-18). 13C NMR (CDCl3, 150 MHz): 37.5, 31.9, 72, 42.5, 140.9, 121.9, 32.1, 32.1, 50.3, 36.7, 21.3, 39.9, 42.6, 56.9, 26.3, 28.5, 56.3, 36.3, 19.2, 34.2, 26.3, 46.1, 23.3, 12.2, 29.4, 20.1, 19.6, 19, 12 ; MS (m/z): 414(M+), 396, 339, 325, 310, 298, 257, 227, 140, 139, 125, 97, 71, 57.

Compound 3 : olean-12-ene-3b,16b-diol isolated from ethyl acetate fraction, mp 221 °C
IR (KBr, cm^{-1}) 3404.55 ,2960 ,2918.36,2852,81,1716.54,1596, 1465 , 1383 ,749.69 ,1059; ^{1}H NMR (CD_3OD, 400MHz) 0.89 (s,H-25) et 0.93(s, H-26), 1.30-1.61 (35H : series of very dense peaks of CH_3, CH_2 and CH) , 2.17 (s ,H-11), 3.09-3.11 (m,H-3) , 3.55-3,66 (m,H-16), 5.50 (s,H-12). 13 C NMR(CD3OD, 125MHZ) : 38.6 (C-1) , 27.7(C-2), 78.9 (C-3) , 38.8 (C-4), 55.4 (C-5), 18.5 (C-6), 32.6 (C-7), 39.7 (C-8), 47.7 (C-9), 36.9 (C-10), 23.7 (C-11), 122.3 (C-12), 144.2 (C-13), 41.7 (C-14), 28.1(C-15), 77.8 (C-16), 46.8 (C-17), 41.3 (C-18), 46.4 (C-19), 31.1 (C-20) , 35.8 (C-21), 32.1 (C-22), 28.4 (C-23), 15.7(C-24), 16.0 (C-25), 16.9 C-26), 26.1 (C-27), 28.5 (C-28) ,33.1 (C-29) , 22.3 (C-30).

Compound 4: 7-O-(α-ramnopyranosyl-(1-6)-β-glycopyranosyl)- 6-Geranyl-5,2',4'-trihydroxy-flavanone , isolated from butanol fraction.
Brown powder, mp 241 C, ESI-MS m/z: 745.3 [M−H]– . UV max (MeOH) : 287 nm, 349 nm, IR (KBr, cm^{-1}) : 3404, 2923, 2852 , 1771 cm-1, 1683 cm^{-1} ,1612, 1459,1388, 1246, 1061,989, 815 ,755 . ^{1}H NMR ($CDCl_3$, 400MHz) δ: 13.10 (1H, br s, 5-OH), 6.32 (d,3.4,H-5'), 7.61 (d,3.4,H-6'), 6.91 ppm (s ,H-3') ,6.12 (s,H-8) , 5.43 (m ,H-2'') , 5.24 (dd ,14.3,3.4, H-2), 5.05 (m,H-6'') , 4.99 (d,3.4,H-1''') ,4.18 (d,J=9.8 Hz), 4.09-4.11 (m, H6'''a, H-6''''b, H-2'''' ,H-5''''), 3.85-3.73 (m, H-2''',H-5''',H-3'''',H-4''''), 3.52 (t,J=9 .8, H-3''',H-4''') ,2.95 (m,H-3) ,2.54(m, H-1''),2.37 (s, Me-3''), 2.18 (m,2H, H-4'' , H-5''), 1.25 (s,6H,9''-Me,8''-Me), 1.15(d,9,Me-5''''). ^{13}C NMR ($CDCl_3$, 125MHz) δ: 74.2 (C-2), 41.8 (C-3), 190.9 (C-4), 105.1 (C-10), 158.4 (C-5), 99.3 (C-6), 165.2 (C-7), 94.5 (C-8), 160.1 (C-9), 110.1 (C-1'), 157.6 (C-2'), 108.3 (C-3'), 163.4 (C-4'), 121.7 (C-5'), 130.2 (C-6'), 28.2 (C-1"), 123.1 (C-2"), 136.0 (C-3"), 16.1 (C-4"), 40.4 (C-5"), 27.5 (C-6"), 125.0 (C-7"), 131.5 (C-8"), 25.7 (C-9"), 18.1 (C-10"). Sugar moiety : 76.2 , 75.45, 72.9 , 71.98 , 70.61, 70.2 , 69.52, 68.23, 65.97 , 17.8.

Compound 5 : 5,7,4' trihydroxy-3-(3 ,6-dimethyl-2,5-heptadienyl) flavones, isolated from butanol fraction, brown powder, ESI-MS m/z: 405.3 IR [M−H]– ^{1}H NMR ($CDCl_3$) δ: 13.13 (1H, br s, 5-OH),: 7.67 (d,7.4, H-2',H-6'),7.46 (d,7.4 H-3',H-5'),7.04 (s,H-6) ,7.00 (s, H-8), 5.30 (s,H-2''), 4.95 (d, 9.1,H-5'') ,2.78 (s ,H-1''). 2.75 (d,3,H-4'') ,1.83(s,6H, 7''-Me, 8''-Me), 1.76 (s,9''-Me). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 161.5 (C-2), 121.6 (C-3), 182.9 (C-4), 105.0 (C-10), 163.3 (C-5), 99.4 (C-6), 65.3 (C-7), 94.3 (C-8), 159.3 (C-9), 24.6 (C-9), 121.7 (C-1'), 128.2 (C-2'), 115.9 (C-3'), 160.3 (C-4'), 115.9(C-5'), 128.2 (C-6') 27.9 (C-1"), 123.2 (C-2"), 136.1 (C-3"), 16.4 (C-4"), 39.6 (C-5"), 27.3 (C-6"), 125.3 (C-7"), 131.1 (C-8"), 25.2 (C-9"), 17.9 (C-10"), .

Compound 6 : 2'',3'',6,8 –Tetrahydroxy-3',4',5',5-tetramethoxy-10,4- cyclolignan, isolated from butanol fraction, A white amorphous powder , MS: m/z 447.3 [M-H] , IR (KBr, cm^{-1}) 3415 , 2924 , 1456 ,1613, 815 , 755 . ^{1}H NMR (CD3OD, 400 MHz): , 6.77 (2H, s , H-2' and H-6'), 3.93 (dd , 5.5Hz, 6.4, H-3" b), 3.88 (s ,4 '-OCH 3),3.82(s,3'-OCH3), 3.83 (s , 5'-OCH 3),3.77(s,5-OCH3) , 3.74(s,7-OCH3), 3.79 (br d , J = 7.1,H-4), 3.54 (dd ,10.0, 5.5Hz, H-3" a), 3.51- 3.46 (3H, m ,H-2"a, H-2"b, H-1b), 2.70 (t, 7.1Hz, H-2), 1.93 (2H, m , H-3,H-1a).

Compound 7: *3,4-Di-O-galloylglucopyranoside* A white powder, isolated from butanol fraction,
MS: m/z: 483 [M-H]. IR (KBr, cm^{-1}) 3404, 2934, 1765, 1612, 1459, 1383, 1257, 1132, 815; 1 H NMR (CD3OD,400 MHz): 7.14 (2H, s, H-2 and H-6); 7.09 (2H, s,H-2' and H-6'), 5.42 (t, 9.6Hz, Glc H-3"), 5.14 (t, 9.0Hz, Glc H-4"), 4.87 (d, 8.8Hz, Glc H-1"), 4.5–3.5 (3H, m, Glc H-2" and 2 H-6"), 4.19 (m, Glc H-5"). ^{13} C NMR (CD_3OD, 125MHz) : 97.80 (C-1"), 74.76 (C-2"), 77.32 (C-3") ,71.86 (C-4"), 79.06 (C-5"), 60.13 (C-6"), 117.49 (C-1'), 108.66 (C-2'), 145.39 (C-3'), 139.91 (C-4'), 145.39 (C-5'), 108.66 (C-6'), 164.82 (C=O'), 118.09 (C-1), 108.56 (C-2), 145.39 (C-3), 139.51 (C-4), 145.39 (C-5), 108.56 (C-6), 165.11 (C=O).

CONCLUSION

The analysis of chemical content of launaea arborescebs allowed us firstly to use various chromatographic techniques and develop a separation process based on the principle of coupling liquid column chromatography and UV spectrophotometer (LC-UV). This technique led to identify a trace compounds. This phytochemical study explain the traditional uses and biological properties of this medicinal plant, it has also demonstrated their value as a source of new natural products that can have a pharmaceutical interest.

Acknowledgment
The authors are grateful to the MESRS – Algeria-, for the financial support.

REFERENCES

[1] Quezel P., Santa S. Nouvelle Flore d'Algérie et des Régions Désertiques Méridionales,CNRS, Paris. vol. 1–2 (**1963**) :1162 p .
[2] OZENDA, P. Flore et Végétation du Sahara. CNRS, Paris,(**2004**) : 662 p.
[3] Belboukhari N , Chériti A. *Pak. J. Biol.Sci*. (**2006**) ; 9(1). 1-2.
[4] Cheriti A, Belboukhari N , Belboukhari M, Djeradi H, *Current Topics in Phytochemistry* , 11 (**2012**), 67-80.
[5] Giner R.M., Diaz J., Manez S., Recio M.C., Soriano C.,Rios J.L. *Biochem System Ecol*.(**1992**): 20, 187–188.
[6] CHERITI A. SAAD A. BELBOUKHARI N. GHEZALI S. *Chemistry of Natural Compounds,* (**2006**) ; 42:360-361
[7] Bitam f., Letizia Ciavatta M., Manzo E., Dibi A., Gavagnin M., *Phytochemistry* (**2008**); 69: 2984–2992
[8] Cheriti A., Belboukhari N., Hacini S., *Ir.J.Pharm.Res*.,(**2004**) ;3 :51.
[9] OZENDA P. Flore du Sahara. Edition ,CNRS : Paris, (**1983**) : 431 p.
[10] Belboukhari N , Chériti A.,Hacini S., Phytochemical and antimicrobial screening of *Launaea arborescens,Seminaire* international sur la valorization des plantes medicinales deans les zones arides, Ouargla: p 7.
[11] Rosset R .,Caude M.,Jardy A., Chromatographie en phase liquide et supercritique, 3éme ED., Masson ,**1991** ;
[12] Cheriti A., Belboukhari N., Hacini S.,EL Abed D. Plante médicinales de la région de Béchar : Teneurs en flavonoïdes et évaluation antibacterienne,Seminaire internationales sur la valorisation des plantes médicinales dans les zones arides, Ouargla, (**2005**) ;P 12.
[13] Hostettman K.,Marston A.,Hastettman M. Preparative Chromatography Techeniques.Applications in Natural Product Isolation .2nd Ed., Springer, Berlin Heidelberg ,**1968**
[14] Lalita M. C., Sonia P., Ireneusz K.,Suranganie F. D., Frances M. S., Peter J. P., Tom J. M. *Phytochemistry* (**2008**);69:961–972
[15] Hisashi K., Noriko S., Akiko H., Haruo O. *Phytochemistry* 29 (**1990**);7: 2351-2355.
[16] González, A. G., E. M. Rodríguez Pérez, C. Hernández Padrón, and J. Bermejo Barrera.. Phytochemical investigation of Canary Island lichens, (**1997**) : 49–60.
[17] Shen XR.,Zhang DZ. *Acad Journal Guangdong coll pharm* (**2006**);22(6):594-5.
[18] ZHANG XIAO P.,PEI YUE H., LIU MING S., KANG SHENG L., ZHANG JUN Q. *Asian pacific journal of tropical medicine* (**2010**) 109-111.
[19] Zhuo C., Yu-Mei L., Song Y., Bao-An S., Guang-Fang X., Pinaki S. B.,Lin-Hong J., De-Yu H., Fang L., Wei X., Xia Z. *Bioorganic & Medicinal Chemistry* (**2008**) ; 16 :1337–1344.
[20] Ongoka P. R., Banzouzi J. T., Poupat C., Ekouya A., Ouamba J. M. and Moudachirou M. *African Journal of Biotechnology* (**2008**); 7 (11): 1727-1730.
[21] Toshihiro A.,Motohiko U., Harukuni T., Hiroyuki S.,Teruo M., Eiichiro I., Ken Y.,Yoshimasa K.,Hoyoku N. *Cancer Letters*,(**2002**); 177: 7–12
[22] Klaus P. L., Maki A., Andreas S., Herbert Kej. *Phytochemistry* (**2008**); 69: 820–826.
[23] Choudhary M.I., Nadra N., Ahmed A., Sajjad A., Atta U.R. *Phytochemistry* (**2009**) ;70: 1467–1473.
[24] Hussein S. A. M., Barakat H. H., Merfort I., Nawwar M. A. M. *Phytochemistry* (**1997**); 45(4): 819-823.
[25] Feng-Lin Hsu ,Li-Ming Yang, Shwu-Fen Chang, Li-Hsuan Wang , Chung-Yi Hsu,; Pan-Chun Liu, Shwu-Jiuan Lin. *Appl Microbiol Biotechnol* (**2007**); 74:659–666.