Journal of Medicinal Plants Research Vol. 5(16), pp. 3758-3762, 18 August, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Antioxidant, antimicrobial activities and fatty acid components of leaf and seed of *Bupleurum lancifolium* Hornem.

**Ali Shafaghat**

Department of Chemistry, Islamic Azad University, Germi Branch, Germi, Iran. E-mail: shafaghata@yahoo.com.

Accepted 14 June, 2011

**The hexane extracts of leaf and seed of *Bupleurum lancifolium* Hornem, which were collected from northwestern Iran, were obtained by Soxhlet apparatus. The fatty acids were derived to methyl esters and determined by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/ mass spectrometry (GC/MS) systems. The hexanic extract from the leaf and seed contained ω-3 (17.1 and 48.1% respectively). The other main components of the leaf and seed extracts were ω-6 (14.1 and 22.2%), palmitic acid (25.7 and10.1%) and γ-linolenic acid (GLA) (21.3 and 8.2%), respectively. The hexane extracts of leaf and seed from *B. lancifolium* detected as an important source of ω-3 and ω-6 compounds among several *Bupleurum* species. The antioxidant activity of both hexane extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate that hexane extracts from different parts of *B. lancifolium* possess considerable antioxidant activity. The highest radical scavenging activity was detected in seed ($IC_{50}$ = 125 µg/ml). The antimicrobial activity of the extracts of those samples were determined against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The bioassay showed that the oil exhibited moderate antimicrobial activity. This study reveals that all parts of this plant are attractive sources of fatty acid components, especially the essential ones, as well as effective natural antioxidants.**

**Key words:** *Bupleurum lancifolium*, Apiaceae (Umbelliferae), fatty acid, antioxidant activity, antimicrobial activity, ω- 3.

## INTRODUCTION

The genus *Bupleurum* with about 180 to 190 species spreading in the North Temperate Zone (mainly in Eurasia, Mediterranean and North Africa), is one of the largest genera of the family Apiaceae (Liu et al., 2003; Neves and Watson, 2004). The Iranian flora comprises fourteen species of *Bupleurum*, among which three are endemic: *Bupleurum flexile, Bupleurum ghahremani and Bupleurum wolffianum* (Mozaffarian, 2007).

**Abbreviations: GC/MS,** Gas chromatography/ mass spectrometry; **GC/FID,** gas chromatography/ flame ionization detector; **GLA,** γ-linolenic acid; **DPPH,** 1, 1-diphenyl-2-picrylhydrazyl; **IOOC**, International Olive Oil Council; **DDM,** disc diffusion method; **PUFAs,** polyunsaturated fatty acids; **ALA** or **ω-3,** α-linolenic acid; **LA,** linoleic acid; **EFAs,** essential fatty acids; **UFA,** unsaturated fatty acid; **SFA,** saturated fatty acid.

Species within *Bupleurum* are easily recognized by the parallel venation of the simple leaves, conspicuous bracts and bracteoles on the inflorescences. Pollen morphology exhibits little variation in this genus. The fruit is oblong to ovoid-oblong or ellipsoid, slightly laterally compressed and the mericarp is subpentagonal (rarely rounded) in cross section. *Bupleurum* is a primitive genus in Apioideae in recent molecular studies (Plunkett et al., 1996a, b, 1997; Downie and Katz-Downie, 1996, 1999; Downie et al., 1998, 2000a, b; Plunkett and Downie, 1999, 2000). Despite intensive research on *Bupleurum*, the phytochemical investigation and fatty acid composition are incomplete.

Traditionally, the woody Araliaceae is regarded as the ancestor of the herbaceous Apiaceae (Calvino et al., 2006). Woody *Bupleurum fruticosum* L. in North Africa is the possible ancestor of the species in genus *Bupleurum*

(Gruas-Cavagnetto and Cerceau-Larrival, 1978). A hypothesis on the origin of Apiaceae suggests that *B. fruticosum* might have migrated northward into Eurasia through the Middle East (Plunkett et al., 1996b). However, Neves and Watson (2004) suggested that the genus *Bupleurum* originated in the western Mediterranean. Although *B. fruticosum* was nested within the western Mediterranean clad, it was not the most basal taxon, thus casting doubt regarding the origin of this genus.

The chemical constituents of the aerial parts of six species of *Bupleurum* genus (*Bupleurum kunmingense, Bupleurum polyclonum, Bupleurum wenchuanense, Bupleurum longicaule var. franchetii, Bupleurum rockii and Bupleurum chaishoui*) medicinally used in south-west region of China have been investigated with the results of obtaining eight flavonoid compounds and six triterpenoid saponins (Luo and Jin, 1991).

A study on the chemical constituents of volatile oils from 19 species of the genus Bupleurum in China has been reported and 131 chemical constituents of those oils were identified by means of gas chromatography/ mass spectrometry (GC/MS) combination. Based on the results of the identification, the authors suggest that the aliphatic compounds could be regarded as the characteristic chemical constituents of the volatile oils of the genus Bupleurum (Guo et al., 1990).

In recent years, increasing studies have been carried out on the essential oils from *Bupleurum* species and the results showed that the root oils possessed significant antifungal (Fernandez et al., 2004), anticonvulsive (Liu et al., 2002) and anti-inflammatory effects (Martin et al., 1993; Ocete et al.,1989; Lorente et al., 1989). The published results revealed that major volatile constituents obtained from *Bupleurum chinense* and *Bupleurum scorzonerfolium* roots were methyl thymol, butylidene phthalide, *α*-terpineol, terpinen-4-ol, *β*-pinene, *α*-pinene, *p*-cymene, (E,E)-2,4-decadienal (Ge et al., 2000; Pang et al., 1992). In a previous investigation on *Bupleurum falcatum*, Zhang et al. (1998) reported that linalool, limonene, *β*-gurjunene, hexanal, (E)-2-nonenal, heptanal, and methyl thymol were the main constituents of the oil. To the best of our knowledge, there is no previous report on the fatty acid composition of the leaf and seed extracts from the *Bupleurum lancifolium* and those biological activities. Therefore it is important and necessary to investigate further the composition of the leaf and seed hexanic extracts and biological activities.

## MATERIALS AND METHODS

### Plant materials

Leaf and seed of *B. lancifolium* were collected separately in the Khalkhal- Ardabil road (Ardabil province) area at an altitude of 1650 m in August 2010. A voucher specimen (B-412) is kept at the Herbarium of Agriculture Research in Ardabil Center, Iran.

### Extraction

Dried and powdered materials (leaf and seed) were extracted with hexane using a Soxhlet apparatus (70°C, 4 h) to obtain the fatty acids and the other apolar components. During extraction procedures, Merck hexane (95%) was used. The extracts were concentrated by rotary evaporator under vacuum at 45°C. The extraction yields were presented in Table 2.

### Methylation of hexane extract

After removing hexane using rotary evaporator, the oily mixtures were derived to their methyl esters by the International Olive Oil Council (IOOC) (2001) and IUPAC (1992) reports by trans-esterification process. In this process, dried hexane extracts were dissolved in hexane and then extracted with 2 M methanolic KOH at room temperature for 30 s. The upper phases were analyzed by gas chromatography/flame ionization detector (GC/FID) and GC/MS systems.

### GC analysis

GC analysis was performed on a Shimadzu 15 A gas chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). $N_2$ was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table 1.

### GC/MS analysis

GC/MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5° C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 1 ml/min. MS were taken at 70 eV. The fatty acids were identified by comparing their retention times and mass peaks with those of standard methyl ester mixtures and by NIST-Wiley library data search. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

### Antioxidant activity tests

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out according to the modified method of Cheung et al. (2003). Briefly, 0.5 ml of DPPH in ethanol (0.1 mM) was added to 1 ml of hexane extract in different concentrations (0.1 to 1.6 mg/ml) and kept in the dark for 10 min.

The absorbance of the resulting solution was recorded on a spectrometer at 520 nm against a blank of hexane. Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as $IC_{50}$ values (µg extract/ ml) for comparison. $IC_{50}$ value of each sample defined as the concentration of sample required for the 50% decrease in absorbance of the blank was calculated.

### Antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the extracts were evaluated by the disc diffusion method (DDM) using Mueller-

**Table 1.** Chemical composition (%) of the hexanic extract from leaf and seed of *Bupleurum lancifolium*.

| Compound* (Related fatty acid) | Rt (min) | % (s) | % (l) |
|---|---|---|---|
| Tetradecanoic acid, methyl ester (myristic acid) | 9.9 | 0.7 | 2.3 |
| Hexadecanoic acid, methyl ester (palmitic acid) | 11.6 | 10.1 | 25.7 |
| 6,9,12-Octadecatrienoic acid, methyl (γ-linolenic acid) | 12.7 | 8.2 | 21.3 |
| 9,12-Octadecadienoic acid, methyl ((linoleic acid) or ω-6 | 12.9 | 22.2 | 14.1 |
| 9,12,15-Octadecatrienoic acid, methyl ester ( linolenic acid) or ω-3 | 13.0 | 48.1 | 17.1 |
| 1,3-Cyclooctadiene | 13.1 | 5.1 | 6.1 |
| Octadecanoic acid, methyl ester (stearic acid) | 13.2 | 3.1 | 4.8 |
| 11-Eicosenoic acid, methyl ester (11-eicosenoic acid) | 14.4 | 0.6 | 0.8 |
| Eicosanoic acid, methyl ester (arachidic acid) | 14.6 | 0.3 | 2.5 |
| Bis(2-ethylhexyl) phthalate[Bis (2-ethylhexyl) phthalic acid] | 16.1 | 0.5 | 4.1 |
| Total (%) | -- | 98.9 | 98.8 |

*The composition of the extracts was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention times (Rt). Rt = Retention time; l = leaf; s = seed.

**Table 2.** Class compositions and yield of the hexanic extract from leaf (l) and seed (s) of *Bupleurum lancifolium*.

| Class composition | l (%) | s (%) |
|---|---|---|
| Essential oil | 0 | 0 |
| Saturated fatty acid | 34.3 | 14.2 |
| Unsaturated fatty acid | 57.4 | 79.6 |
| Other compounds | 6.1 | 5.1 |
| Yield | 2.8 | 3.6 |
| UFA/SFA*: | 1.6 | 5.6 |

*UFA = Unsaturated fatty acid; SFA = Saturated fatty acid.

Hinton agar for bacteria and Sabouraud dextrose agar for fungi (Baron and Finegold, 1990). Discs containing 30 µl of the hexanic extracts were used and growth inhibition zones were measured after 24 h and 48 h of incubation at 37 and 24°C for bacteria and fungi, respectively. Gentamicin and tetracycline for bacteria and nystatin for fungi were used as positive controls. The microorganisms used were: *Bacillus subtilis* ATCC 9372, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 3583, *Pseudomonas aeruginosa* ATCC 27852, *Escherichia coli* ATCC 25922, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 5027 and *Saccharomyces cerevisiae* ATCC 9763.

## RESULTS AND DISCUSSION

The results obtained in the analyses of the hexane extract of *B. lancifolium* leaf and seed are listed in Table 1, in which the percentage and retention time of components are given. According to the results, the hexane extract yields of the studied different part of *B. lancifolium* were found 2.8% (leaf extract) and 3.6% (seed extract) on the basis of dry weight of the plant materials. The highest total percentage was detected in seed. The total fatty acid contents of hexane extracts varied from 98.8 to 98.9% (Table 1). The major saturated and unsaturated components including linolenic (ω-3), linoleic (ω-6) and palmitic acids are shown in the table. The major polyunsaturated fatty acids (PUFAs) were α-linolenic and linoleic acids.

As can be seen in Table 1, about 98.9% (10 components) of the extract from seed, and 98.8% (10 components) from leaf extract were identified. There were some differences in the fatty acid profiles of the different part of this plant. The unsaturated fatty acid contents were higher than saturated ones, whereas some of the fatty acids were not observed in all parts of this plant. In fact, both fractions mainly include unsaturated fatty acids, with a clear predominance of α-linolenic acid (ALA or ω-3), linoleic acid (LA) and oleic acids. One of the essential fatty acids (EFAs), ω-3 (ALA) was a predominant component in seed of *B. lancifolium*. Linolenic acid is an omega-3 fatty acid, ranging from 17.1 (in leaf) to 48.1% (in seed) in this work. The ratios of unsaturated fatty acid (UFA)/ saturated fatty acid (SFA) were 5.6 and 1.6 in extract from seed and leaf, respectively (Table 2).

**Table 3.** DPPH free radical scavenging activity of hexane extracts of different parts and standard antioxidant, vitamin C.

| S/N | Sample (Hexanic extract) | $IC_{50}$ (µg/ml) |
|---|---|---|
| 1 | leaf | 435 |
| 2 | seed | 135 |
| 3 | Vitamin C (Ref.) | 27 |

**Table 4.** Antimicrobial activity of the hexane extracts of leaf (l) and seed (s) of *Bupleurum lancifolium*.

| Microorganism | Zone of inhibition (mm)** | | | | |
|---|---|---|---|---|---|
| | Hexane extracts | | Antibiotics | | |
| | l* | s* | Gentamicin | Nystatin | Tetracycline |
| *B. subtilis* | 18.7±0.4 | 16.3±0.2 | NT[b] | NT | 22±0.2 |
| *S. epidermidis* | 13.6±0.3 | 12.7±0.1 | NT | NT | 34±0.7 |
| *E. faecalis* | 8.3±0.1 | 9.7±0.3 | NT | NT | 9±0.8 |
| *S. aureus* | 13.5±0.1 | 13.9±0.2 | NT | NT | 21±0.6 |
| *K. pneumoniae* | NA[a] | NA | 20±0.4 | NT | NT |
| *P. aeruginosa* | NA | 8.6±0.3 | 11±0.3 | NT | NT |
| *E. coli* | 14.5±0.1 | 16.0±0.4 | 24±0.8 | NT | NT |
| *A. niger* | NA | 8.6±0.2 | NT | 16±0.7 | NT |
| *C.albicans* | 12.5±0.5 | 14.2±0.2 | NT | 18±0.2 | NT |
| *S. cerevisiae* | 11.9±0.4 | 14.7±0.3 | NT | 18±0.4 | NT |

*l: leaf, s: seed ; [a] NA: Not active; [b] NT: Not tested. **Inhibition zone diameter (mm), including diameter of sterile disk 6 mm; values are given as mean ± SD.

Seed of *B. lancifolium* had a higher proportion of UFA compared to leaf part. Hexadecanoic acid, methyl ester was detected in a high amount in leaf (25.7%) as an ester derivatives obtained from extract of this plant. Some hydrocarbon compounds were found in leaf (6.1%) and seed (5.1%) (Table 1).

The antioxidant activity of hexane extracts was also reported for the first time. Results obtained in the antioxidant study of the samples are shown in Table 3. Antioxidant activity was tested according to the DPPH radical scavenging method. The both oils from leaf and seed obtained from different parts of *B. lancifolium* scavenged the DPPH radical in a dose-dependent manner, and the DPPH radical scavenging activity ($IC_{50}$) was decreased in the following order: seed > leaf (Table 3).

According to this data, seed was the most efficient free radical scavenger by the lowest $IC_{50}$ value of 135 µg/ml among both the hexane extracts. The activity of the reference antioxidant (vitamin C) was much higher than that of seed oil. Although seed oil did not differ considerably in fatty acid composition, it exhibited the best DPPH scavenging activity.

The extracts of leaf and seed from *B. lancifolium* was tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The results, presented in Table 4, show that the hexane extracts exhibited moderate biological activity against all tested fungi and bacteria except for two resistant Gram-negative bacteria, *K. pneumoniae* and *P. aeruginosa*, as well as a fungi, *A. niger.* The most sensitive microorganisms were *B. subtilis* and *E. coli*, with inhibition zones of (16.3 to 18.7) and (14.5 to 16) mm, respectively. Other microorganisms were found to be less sensitive to the extracts with inhibition zones ranged from 8 to 14 mm. It is conceivable that the antimicrobial property of the hexane extracts from *B. lancifolium* might be ascribed to its high content of fatty acids.

## ACKNOWLEDGMENT

The author wish to thank Iran National Science Foundation (INSF) for financial support for this investigation.

## REFERENCES

Baron EJ, Finegold SM (1990). Methods for testing antimicrobial effectiveness. In: Diagnostic Microbiology. Stephanie M (Ed.). Baltimore, Mosby, pp. 171-194.

Calvino CI, Tilney PM, Wyk BV, Downie SR (2006). A molecular phylogenetic study of southern African *Apiaceae*. Am. J. Bot., 93: 1828-1847.

Downie SR, Katz-Downie DS (1996). A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot., 83: 234-251.

Downie SR, Katz-Downie DS (1999). Phylogenetic analysis of chloroplast rps16 intron sequences reveals relationships among woody southern African Apiaceae subfamily Apioideae. Can. J. Bot., 77: 1120-1135.

Downie SR, Katz-Downie DS, Watson MF ($2000_a$). A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA rpl16 and rpoC1 intron sequences: towards a suprageneric classification of subfamily Apioideae. Am. J. Bot., 87: 273-292.

Downie SR, Watson MF, Spalik K, Katz-Downie DS ($2000_b$). Molecular systematic of Old World Apioideae (Apiaceae): relationships among some members of tribe *Peucedaneae sensulato*, the placement of several island-endemic species, and resolution within the apioid superclade. Canad. J. Bot., 78: 506-528.

Downie SR, Ramanath S, Katz-Downie DS, Lianas E (1998). Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid rpoC1 intron sequences. Am. J. Bot., 85: 563-591.

Fernandez OAM, Gomez RMV, Velasco-Negueruela A, Camacho SAM, Lopez CF, Altarejos J (2004). *In vivo* antifungal activity of essential oil of *Bupleutum gibraltarium* against *Plasmopara halstedii* in sunflower. J. Agric. Food. Chem., 52: 6414-6417.

Ge HF, Li Y, Xie JM, Li J, Ma GJ, Chen YH, Lin YC, Li XF (2000). Studies on technology of supercritical-CO, fluid extraction for volatile oils and saikosaponins in *Bupleurum chinense* DC. J. Chin. Mater. Med., 3: 149-152.

Gruas-Cavagnetto C, Cerceau-Larrival MT (1978). Presence of fossil pollen of Umbelliferae in the Paleogene of the Anglo-Parisian Basin: Miss. Bot. Gard., 255–267.

Guo J, Pan S, Li Y, Hong X, Wang Z (1990). A study on the chemical constituents of volatile oils from 19 species of the genus *Bupleurum* in China. J. Shang. Med., 4: 8-12.

Liu LH, Dao ZL, Guo HJ, Li H (2000). Flora of Gaoligong Mountains In: Li-H, Guo H-J, Dao Z-L eds. 2001. Flora of Gaoligong. Beijing: Science Press. pp. 1-46.

Liu Y, Wu H, Ge F (2002). Chemical constituents analysis on anticonvulsive effect of three extracts from *radix bupleuri*. Zhon. Ya. Ca., 25: 635-637.

Lorente I, Ocete MA, Zarzuelo A, Cabo MM (1989). Comparative study of different essential oils of *Bupleurum fruticosum*. J. Nat. Prod., 52: 267-272.

Luo S, Jin H (1991). Chemical constituents of the aerial parts of six species of *Bupleurum* genus medicinally used in south-west region of China. Zhong. Zhon. Ya. Za Zh., 16(6): 353-356.

Martin S, Padilla E, Ocete MA, Galvez J, Jimenez J, Zarzuelo A (1993). Anti-inflammatory activity of the essential oil of *Bupleurum fruticescens*. Planta Med., 59: 533-536.

Mozaffarian V (2007) A Dictionary of Iranian Plant Names. Farhang Moaser Publishers, Tehran, Iran. pp. 86-87.

Neves SS, Watson MF (2004). Phylogenetic relationships in *Bupleurum* (Apiaceae) based on nuclear ribosomal DNA ITS sequence data. Ann. Bot., 93: 379-398.

Ocete MA, Risco S, Zarzuelo A, Jimenez J (1989). Pharmacological activity of the essential oil of *Bupleurum gibraltaricum*: anti-inflammatory activityand effects on isolated rat uteri. J. Ethnopharmacol., 25: 305-313.

Pang JH, Yang B, Liang WS, Jia Q, Zhang RY (1992). Analysis compositions of essential oil from *B. Scorzonerifolium* Willd by GC-MS. J. Beijing Med. Univ., 2: 501-502.

Plunkett GM, Downie SR (2000). Expansion and contraction of the chloroplast inverted repeat in Apiaceae subfamily Apioideae. Syst. Bot., 25: 648-667.

Plunkett GM, Downie SR (1999). Major lineages within Apiaceae subfamily Apioideae: a comparison of chloroplast restriction site and DNA sequence data. Am. J. Bot., 86: 1014-1026.

Plunkett GM, Soltis DE, Soltis PS (1996a). Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of race sequences. Am. J. Bot., 83: 499-515.

Plunkett GM, Soltis DE, Soltis PS (1996b). Evolutionary patterns in Apiaceae: inferences based on matK sequence data. Syst. Bot., 21: 477-495.

Zhang Y, Fu CH, Xiang YC, Li P, Ming XL (1998). Analysis compositions of essential oil from *Bupleurum falcatum* L. by GC-MS. J. Cheng Du Univ. TCM, 21: 51-52.