Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.9 No.4, 2017

Phytochemical Analysis, Isolation and Identification of Flavan-3ol from Syrian Pinus Halepensis

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Abstract
The present study is carried out with steam bark of *pinus halepensis* : A simple and fast method was developed for flavan-3ol from steam bark of *pinus halepensis* using by flash column chromatography then purification were conducted by preparative chromatography The structures of these compound were characterised by comparing their spectral data including 1D and 2D NMR with those reported in the literature The qualitative phytochemical analysis revealed that, extracts of (green dry parts from plant) contain tannins, flavonoids, terpenoids, alkaloids as well as steroids. phenolic flavonoids, the molecular structures have determinate by spectroscopy methods :FT-IR, MS, 1H-NMR, 13C-NMR, DEPT (90,135), HMQS, COSY and HMBC.
Keywords: *pinus halepensis*, flavan-3ol, Phytochemicals, NMR.

1- Introduction

The genus Pinus belongs to the family Pinaceae and comprises about 250 species. It is the largest genus of conifers occurring naturally in the northern hemisphere, especially in the Mediterranean region, Caribbean area, Asia, Europe, North and Central American [1-2]. The genus Pinus has been planted in the temperate regions of the southern hemisphere. They are evergreen and resinous trees growing to 3-80 m tall with needle-like gray-green leaves that grow in pairs

Taxonomy: Kingdom : Plantae, Division : Pinophyta, Class : Pinopsida, Order : Pinales, Family : Pinaceae, Genus : Pinus Species : halepensis, Binomial name: *Pinus halepensis*

The medicinal and aromatic properties of the chemical compounds (*e.g.*, turpentine, resins and essential oil….) of pine make it one of the most popular plants throughout all civilization. Pine is also still widely used in traditional therapeutic practice in world and has economic importance,[3-4]. In the Northern Mediterranean basin, *Pinus halepensis* Miller (*P. halepensis*) is a pioneer and expansionist species that colonizes abandoned agricultural lands characterized by high biodiversity, Several phytochemical analyses of *P. halepensis* have been published on terpenes[5-6], turpentine[7] and phenolic compounds[8] .

This study describes the isolation and structural determination of the flavan-3-ol(afzelechin) from *pinus halepensis* from syria.

Figure 1. photo of *pinus halepensis*.

2- Experimental

2.1. Materials and Methods:

Melting points were measured on an Electrothermal Entineering melting point apparatus /LTD/ spectrum NMR proton and carbon device 400 MHz model Bruker by Switzerland company, optical absorption spectrum infrared device model FT-IR-4100 from the Japanese company Jasco, rotary evaporator 4.91 model from the German company Normschiff, thin layer chromatographic of aluminum coated by Silica Gel 60F254 measuring 20 X 20 from the German company Merck, thin layer chromatographic of preparatory glass coated by Silica Gel 60F254 measuring 20 X 20 from the German company Merck, silica gel (230 – 400 mesh, ASTM).

2.2. Chemicals solvents
The solvents like ethanol (95%), petroleum ether, benzene, ethyl acetate from Merck (India).
Plant materials
The steam bark of *pinus halepensis* were collected from Homs in Syria, The plant materials were dried in shade separately.

2.3. Qualitative phytochemical analysis of plant extracts.[9-10]**:** The steam bark extracts were analyzed for the Flavonoids, Pholabatannins, Resins, Tannins, Phenols, Carbohydrates as follows .
2.3.1. Flavonoids (Shino or Pew test): 0.5 g of the extract was dissolved in 2 mL of ethanol and treated with few drops of conc. HCl and 0.5 g of magnesium. The pink colour was observed.
2.3.2. Resins: 0.5 g of the extract was dissolved in 2 mL of ethanol in a test tube and treated with 2 mL of distilled water and observed for turbidity
2.3.3. Tannins: 0.5 g of the extract was dissolved in 2 mL of ethanol and added with 3 mL of hot distilled water and then filtered. Few drops of FeCl3 (0.1 g/dL) were added and allowed to stand for some time and observed for brownish green or blue black colour.
2.3.4. Phenols: 0.5 g of the extract was dissolved in 2 ml of ethanol and added with water. To this 2 ml of FeCl3 was added and observed for the formation of green or blue colour.
2.3.5. Carbohydrates (Molish test): The extract (0.5 g) was dissolved in 2 mL of ethanol and added with 1 mL of distilled water and filtered. To this solution, 2-3 drops of α-naphthol were added followed by 1 mL of H2SO4. The formation of violet coloured ring was observed at the interface of two layers.
Extraction and separation of crude sample
The dried samples 250 gr from the leaf and fluffy parts from trees were chopped into small pieces and extracted with methanol: water (80:20) by soaking for 48 Hrs at room temperature (25°C).

The methanolic extract was decanted, filtered under vacuum, concentrated in a rotary evaporator (40°C.). The resulting crude extract from the leaf and fluffy parts from trees (9 g), suspended in H2O and partitioned successively with petroleum ether ,Dichloromethane, EtOAc and n-buthanol using liquid-liquid extractor to give EtOAc(1.8 g). EtOAc extract was subjected to column chromatography on silica gel. The column was eluted with a gradient of chloroform: methanol (100:0 to 0:100%) to yield several Sub-fractions .(Fractions Ac) 10-30 (10% methanol in chloroform) were mixed due to their similar TLC.

Sub-fraction Ac (300 mg) was dissolved in minimal methanol and subjected to column chromatography on silica gel and eluted with a gradient of chloroform:methanol (100:0 to0:100).. similar fractions based on TLC and observed under UV ligh 245nm)t were combiened.

Sub-fractions .(Fractions Ac1-94mg) (15% methanol in chloroform) was subjected to PTLC to provide afzelechin (21mg- Rf 0.63 chloroform :methanol: water 7:3:0.2).It is a white needles (methanol), m.p. 225°C,

4. Results and discussion
The results of Qualitative phytochemical Contain the antenna portion of the plant on Alkaloids, Resins, Saponins, Flavonoids, Glycosides, Tannins

Table 1. Results of phytochemical analysis of *pinus halepensis*.

Test	Stem extract
Alkaloids	+
Resins	+
Saponins	+
Flavonoids	+
Glycosides	+
Tannins	+

+ = Present - =Absent

4.1. structure identification of the afzelechin
Mass spectroscopy revealed molecular ion peak **at** m/z=274(M+) for C15H14O5 and base peak **at** m/z=108 **for [M-C9H10O3]** (Figure.1)

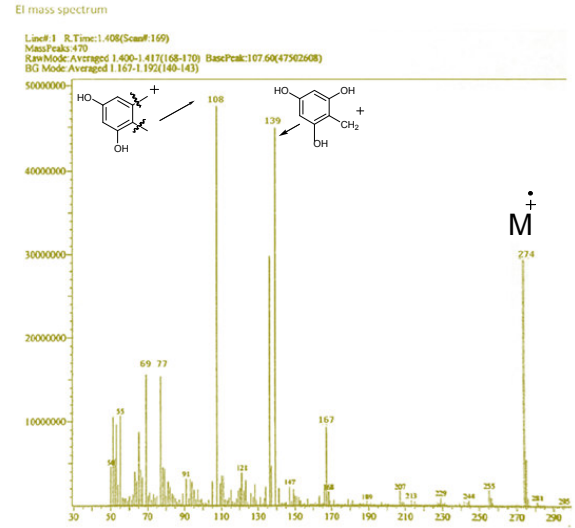

Figure 1. EI-MS spectrum of Flavan-3ol.

The structural determination of the afzelechin based on the usual spectral methods. The FT-IR spectrum shows a broad band at 3357.73cm^{-1}indicating the presence of hydroxyl group in the structure, CH and CH2 str. At 2920.73 cm-1, Ar. C=C bend at 1615 cm-1, , C-O str. at1270 cm-1, 1012 cm-1 , CH bend at 813 -654 cm-1.

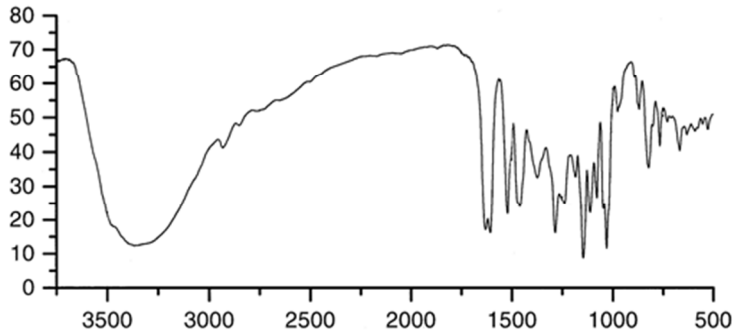

Figure 2. FT-IR spectrum of Flavan-3ol.

Table 1. The absorption of the main factions of the compound.

Functional groups	C-H Aromatic bent	C-O str	Ar. C=C bend	C_{SP}^{3}–H	-OH
Absorbance **""cm^{-1}**	813	1270	1615	2920, 2855	3357

The 13C NMR (Figure 3) spectrum showed the presence of three aliphatic (CH2 x 1, CH x 2, C-O-H x 1) and 12 aromatic (CH x 6, C x 3, C-O x3) signals. The DEPT spectrum (Figure.4) revealed the presence of one methylene, six methine (two of double intensity) and six quaternary carbon atoms.[11]

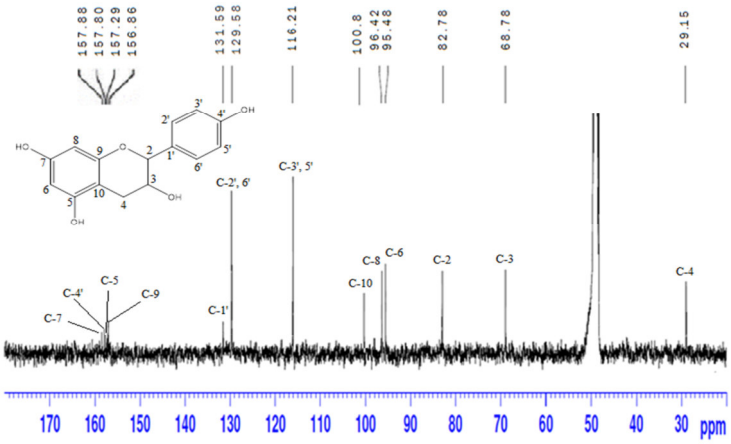

Figure 3. 13C-NMR Spectrum of afzelechin. (125 MHz, CD_3OH)

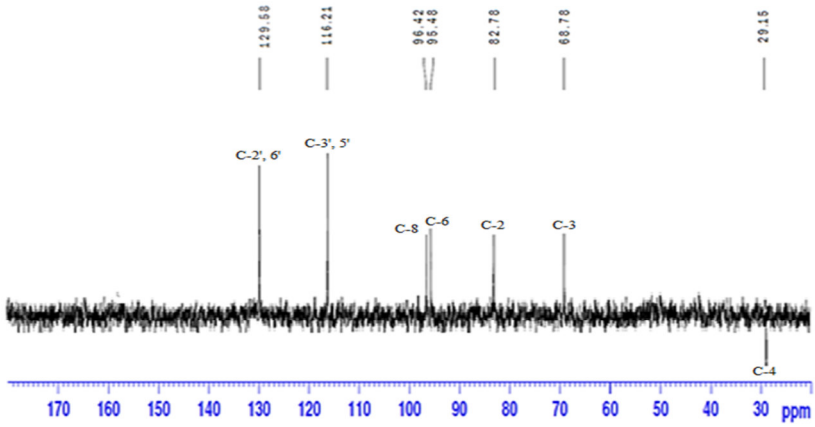

Figure 4. DEPT- 135 Spectrum of **afzelechin**.

The 1H NMR spectrum exhibited characteristic signals for two highly shielded meta coupled aromatic protons [δH 5.83 and 5.92 (each d, J 2.2 Hz)], and a pair of para-disubstituted aromatic protons [δ 7.21 (d, J 8.5 Hz) and 6.77 (d, J 8.5 Hz)]. The proton signals at δ 4.58 (d, J 8.0 Hz), 3.97 (m), 2.87 (dd, J 5.5, 16.1 Hz) and 2.49 (dd, J 8.5, 16.1 Hz) were characteristic of a flavan C ring of a flavan-3-ol skeleton with a phloroglucinol pattern for ring A [δ 5.91 (d, J 2.1 Hz) and 5.83 (d, J 2.1 Hz **[12-13]** see **Figure 5** (Table 1)

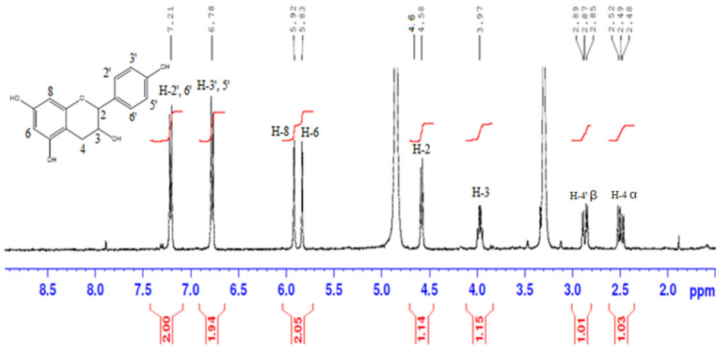

Figure 5: 1H-NMR Spectrum of afzelechin. (400 MHz, CD_3OH, δTMS = 0 ppm)

Table 2. chemical shift (1H-NMR and 13C-NMR) of the compound.

N0	δH (ppm)	δC (ppm)
2	4.58 *d* (8.0	82.77
-3	3.97 *m*	68.87
4	2.87 *dd* (5.5, 16.1) 2.49 *dd* (8.5, 16.1)	29.15
5		157.88
6	5.83 *d* (2.1)	95.48
7		157.8
8	5.92 *d* (2.1)	96.41
9		157.28
10		100.8
1`		131.5
2`	7.21 *d* (8.5)	129.58
3`	6.77 *d* (8.5)	116.21
4`		156.86
5`	6.77 *d* (8.5)	116.21
6`	7.21 *d* (8.5)	129.58

we can indicate from the COSY spectrum (Figure 6), the presence of one (spin – spin) scaling coupling system between the proton at (δH =7.21 ppm, H-`6) and the proton at (δH =6.77ppm, H-`5), and two meta-coupling protons of H-6 and H-8(_ 5.83 and 5.92, each doublet, J=2.1Hz).
The proton at ((δH =4.58 ppm, H-2) coupling with proton at ((δH =3.97 ppm, H-3).
The proton at (δH =2.87 ppm, H-4β) coupling with proton at (δH =2.49 ppm, H-4α),and the proton (δH =3.97 ppm, H-3) coupling with protons at (δH =2.49 ppm, H-4α and δH =2.87 ppm, H-4β).

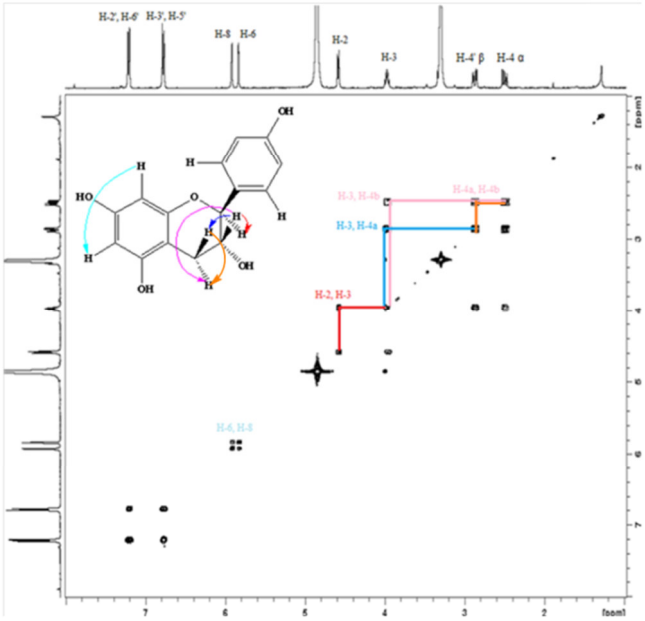

Figure 6: 1H-1H COSY Spectrum of afzelechin.

we determinate from the HMQC spectrum, the heteroatom correlations between hydrogen systems and the carbon atoms carrying these protons **Figure 7**

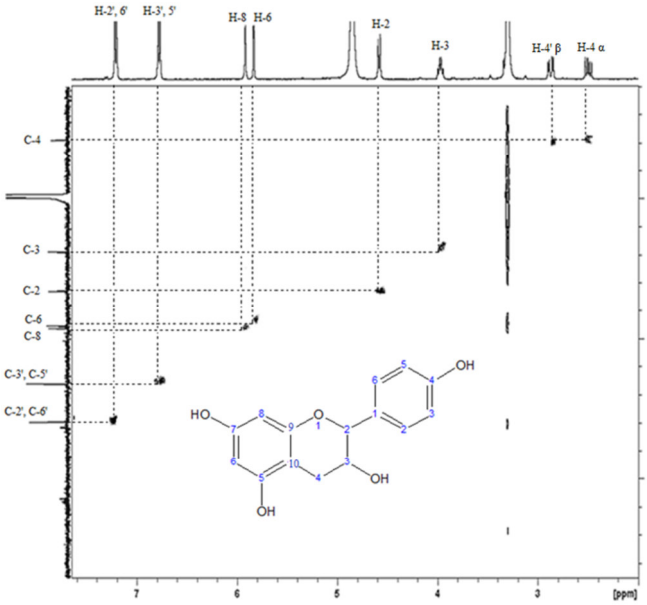

Figure 7: HMQC Spectrum of afzelechin.

The 1H-13C long range coupling information obtained from the heteronuclear multiple bond connectivity (HMBC) experiment allowed the rings to be connected (Figure9). The proton peak at δH 2.87 (H-4) correlated with δC 68.87 (C-3), δC 157.88(C-5), δC 152.28 (C-9), δC 100.8 (C-10) and the proton peak at δH 4.58 (H-2) correlated with δC 68.87 (C-3), δC 157.28 (C-9), δC 131.5 (C-`1) and δC 159.58 (C-`2, `6).

The coupling constant (*d*, *J* 8.0 Hz) for δH 4.58 indicated a flavan H-2 signal with a 2,3-*trans* configuration and this was confirmed by the chemical shift appearance of a signal at δ 82.7 (C-2) ..see table 2, (Figure8)

HMBC data of compound table 3:

Table 2. chemical shift (HMBC) of the compound.

N0	HMBC
2	C-3, C-9, C-1`, C-`2, C-6`
4	C-3, C-5, C-9, C-10
6	C-5, C-8
8	C-6, C-9, C-10
`2	C-2, C-`6, C-`4
`5	C-`1, C-`4, C-`3
`3	C-`1, C-`4, C-`5
`6	C-2, C-`2, C-`4

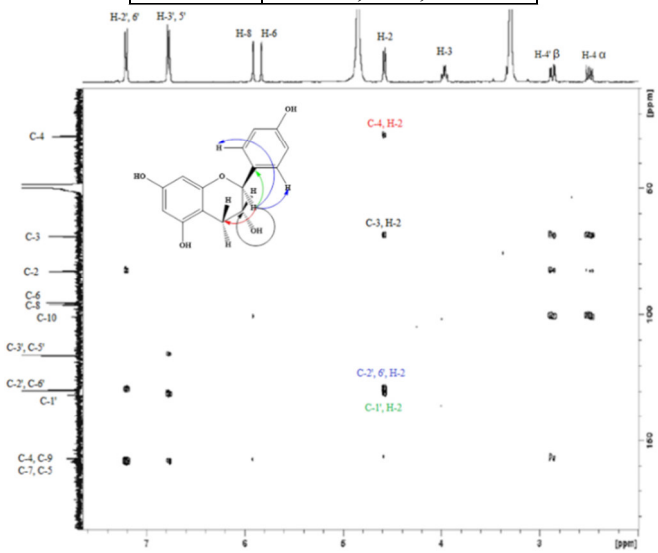

Figure 8. HMBC correlations spectrum of Flavan-3ol.

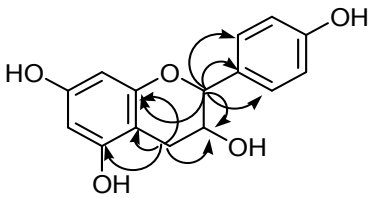

Figure 9. The main structure of compound.

5. Conclusion

The study Phytochemical and isolation and characterization of a compound Flavan -3-ol (Afzelechin) well was to prove his intention roads unilateral spectral and two-dimensional ... and this compound isolated for the first time from the plant *pinus halepensis*.

6. Acknowledgments

The authors express their thanks to central organic laboratory in department of chemistry, AL Baath University, faculty of sciences, for their assistance during the work.

7. References

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