

CHROMATOGRAPHIC IDENTIFICATION OF SOME VOLATILE OILS FROM THE OF PINUS BRUTIA TEN. GROWING IN IRAQ ⁺

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Abstract:

Non – wood forest products are an important part of the forestry industry in the world. The extractives from softwoods contain all classes of terpenes, one of these classes are the volatile oils. This study concentrates on the presence of these oils in the bark of *Pinus brutia* grown in north of Iraq. The Pet.ether (40-60 C°) extract of the bark identified by thin-layer chromatography technique using a plates of (2mm) thickness . By using the silica gel the identification results appeared that, the bark contained at least four compounds (α -pinene, β -pinene, camphene and borneol). These compounds were identified through comparisons with their R_F-values in the tables. The percentage of these components were approximately 3.1% from the air dried bark of *Pinus brutia*.

المستخلص:

تلقي منتجات الغابات الثانوية (عدا الخشب) اهتماما كبيرا في مصانع الغابات في العالم. ان مستخلصات انواع الاخشاب الرخوة تحتوي على جميع اصناف التربينات وان الزيوت المتطايرة واحدة منها. تركزت هذه الدراسة حول وجود هذه الزيوت في قلف صنوبر بروتيا النامي في شمال العراق. ان مستخلص الايثر البترولي (٤٠-٦٠ م°) الذي تم الحصول عليه من القلف ، شخصت مكوناته باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة على الواح بسمك (٢ ملم) المغطاة بالسيليكا جيل. حيث اظهرت نتائج التشخيص احتواء مستخلص القلف على الأقل أربعة زيوت متطايرة (الفابنين ، بيتابنين ، كامفين والبورينيل) شخصت هذه المركبات بالمقارنة مع قيم الـ R_F-values في الجداول. شكلت هذه المكونات حوالي ٣,١% من وزن القلف المجفف هوائيا.

Introduction:

Volatile or essential oils [1] , as their name implies, are volatile in steam. They are differ entirely in both chemical and physical properties from fixed oils. With the exception of oils such as oils of bitter almonds, which are produced by the hydrolysis of glycosides, these oils are contained largely as such in the plant. They are secreted in oil cells, in secretion ducts or cavities or in glandular hairs. They are frequently [2] associated with other substances such as gums and resins and themselves tend to resinify on exposure to air.

Large quantities [3] of volatile oil are produced annually; for 1987, for example it is estimated that the total world production, in metric tons, was: for lemon oil 3000, for eucalyptus oil 2500, for clove leaf oil 2000, for peppermint 6000. Volatile oils are

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used for their therapeutic action, for flavoring, in perfumery or as starting materials for the synthesis of other compounds. Those oils with a high phenol content, have antiseptic properties, whereas others are used as carminatives. Oils showing antispasmodic activity, and much used in popular medicine, are those of *Melissa officinalis*, *Rosmarinus officinalis* and *Citrus aurantium*.

Rectified oil of turpentine [1] is the volatile oil distilled from the oleoresin of pines, it is soluble in alcohol, ether, chloroform and glacial acetic acid. Volatile oils are optically active, but the rotation varies not only with the species of pine from which it has been obtained, but also in samples taken from the same tree at different periods. The oil consists chiefly of the terpenes (+)- and (-)- α -pinene, (-)- β -pinene and camphene.

After wood, the bark [4] is the second most important tissue of a trunk. It amounts to about 10-20% of a stem depending on the species and on growing conditions [5]. The molecular weight of poly phenolic acids from loblolly pine bark (*Pinus taeda*) [6] is in the range of 1500-6700. The poly phenols in the hot water extracts of barks from *Picea abies* and *Pinus brutia* [3] have a molecular weight (MW) of 2700 and 3800 respectively.

Bark is more acidic than wood because of the higher content of acidic compounds. The determined pH values [7] of most southern pines ranging from about 3.1-3.8, with very little differences between species.

Gum oleoresin processing [8] is mainly by the steam distillation process, the batch process is suitable for plant with annual capacity of 1,000-2,000 tons, and the continuous process is used for plant with capacity of more than 3,000 tons per year. The aim of this study is to obtain the volatile oils from the bark of *Pinus brutia* Ten. which is one of the main species grown in the north part of Iraq and to identify the chiefly terpene compounds present in the Pet. Ether extract.

Material and Methods:

1. A crude pet. ether (40-60) C° extract [9]

The dry brown bark (500 gm) was air-dried and then put through a hammer mill using a screen to get 40-mesh and extracted with pet.ether (40-60) C° in a Soxhlet extractor for 3 days. After no pigment was noticed, the pet.ether extract was concentrated under vacuum to give a (9.4 gm) of a crude extract with pale yellow oleoresin.

2. The preparative [10] Silica gel (TLC) plates:

The plates were prepared with 0.2 mm thickness. Before the use, heating carried out for an hr. in an oven at 120 C° and allowed to cool before using.

After completion of run in the Jar, the plates were air dried and the compounds were localized by spraying the plates with Vanillin-H₂SO₄ or exposed to iodine vapor.

3. Chromatographic spray reagents

(i) Vanillin [9]-H₂SO₄

The reagent was prepared by adding 8 ml of methanol to 0.5 gm vanillin in 2 ml conc.H₂SO₄ with cooling. After the development of the chromatogram, spraying was carried out with this reagent to localize the spots (e.g. terpenes) which appeared as brown color spots.

(ii) Iodine^[10] vapor.

The iodine crystals were placed in a covered tank (the tank warmed to increase vaporization). Many compounds absorb iodine reversely to produce yellow to brown spots or faint yellow background.

4. Extraction [9] and identification of α -pinene (1) β -pinene (2) and camphene (3).

1 μ m of the concentrated pet.ether extract (oleoresin) was dissolved in ether and then chromatographed one-dimensionally in silica gel in two solvent systems (Hexane and cyclohexane) for α -pinene, β -pinene and camphene.

Both systems gave four spots with R_f (0.83, 0.78, 0.85, 0.90) for the former and (0.84, 0.80, 0.78, 0.92) for the latter, which correspond to α -pinene(1), β -pinene (2) and camphene (3), the fourth spot is still unknown.

All spots were visualized as brown spots using Vanillin- H_2SO_4 as spraying reagent and yellow spots, using iodine vapor. (Table 1, Fig. 1).

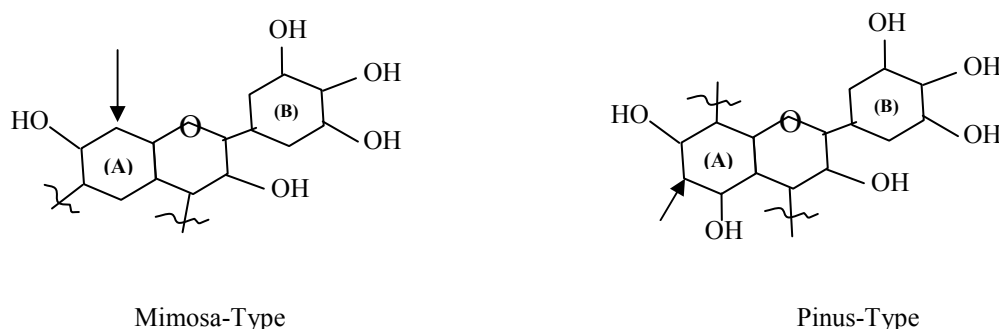
5. Extraction [9] and identification of Borneol(4).

Another run was carried out, using 5% ethyl acetate in benzene as solvent system to identify borneol (4) as green spot ($R_f = 0.19$) using vanillin- H_2SO_4 and yellow spot from iodine vapor. Another spot ($R_f = 0.40$) is still unknown. (Table 2, Fig. 2).

Results and Discussion:

The bark of *Pinus spp.* appeared to contain several compounds, like tannins [11] (hydrolysable and condensed) also oleoresin was present as major active constituent of the pine bark.

Condensed tannins [11] were classified after their hydroxyl-groups pattern in different classes, two groups were recognized deriving from the structure of the A-rings, first one the mimosa or wattle type and the second one the pinus-type.



Contamination [11,12] was occurred when these mentioned compounds are presented in the extracts, thus we used pet.ether as solvent to prevent the overlap between these tannins and the oleoresin content.

Rectified oil of turpentine [1] is the volatile oil distilled from the oleoresin of pines which is afterwards rectified. Rectified oil of turpentine was a colorless liquid

with a characteristic odor and pungent taste, soluble in alcohol, ether, chloroform and glacial acetic acid.

As a result of the phytochemical screening of *Pinus heterophylla*, the same of *Pinus palustris* except the specific rotation of the compounds, oil of turpentine consist chiefly of the terpenes; α -pinene (1), β -pinene (2) and camphene (3).

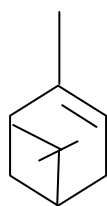
In a continuation of this work, we have identified four volatile compounds by using TLC-plates on silica gel G of pet-ether (40-60) C^o extract which visualized by using chromatographic spraying reagents (Vanilin-H₂SO₄ and Iodine vapor).

Table (1) [10]: R_f-values of Some Terpene

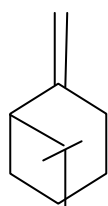
Volatile Oils	R _f in Solvent		Colour in Vanillin H ₂ SO ₄
	Hexane	Cyclohexane	
α -Pinene	0.83	0.84	Brown
β -Pinene	0.80	0.80	Brown
Camphene	0.84	0.79	Brown
Pet.ether ext.	0.83	0.84	Brown
	0.78	0.80	Brown
	0.85	0.78	Brown
	0.90	0.92	Brown

Table (2) [10]: R_f-values of Some Terpenes Alcohols

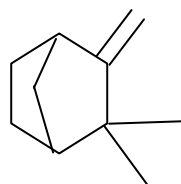
Volatile Oils	R _f in 5%ethylacetate in benzene	Color in Vanillin H ₂ SO ₄
Borneol	0.20	Green
Linalool	0.30	Green
Citronellol	0.27	Green
Pet.ether ext.	0.19	Green
	0.40	Green



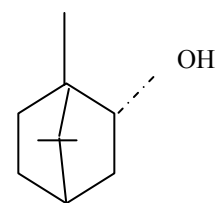
α -pinene (1)



β -pinene (2)



Camphene (3)



Borneol (4)

Chromatographic identification is carried out by using three solvent systems; the first solvent is hexane, the run gave four spots (R_f 0.83, 0.78, 0.85 and 0.90), the second solvent is cyclohexane and the run gave also four spots (R_f 0.84, , 0.82, 0.78 and 0.92). All the spots were visualized as brown spots by using Vanilin-H₂SO₄ and yellow colours by using iodine vapour.

Through comparisons with R_f -values in the tables, we identified at least three compounds α -pinene(1), β -pinene(2) and camphene(3), the four spot is still unknown. (Table 1, Fig. 1).

Moreover, another run is carried out using 5% ethylacetate in benzene as a third solvent to identify borneol(4), (R_f 0.19) which appeared as green spot by using vanillin- H_2SO_4 and yellow spot from iodine vapour.

Another spot appeared also with R_f 0.40 is still unknown. (Table 2, Fig. 2). The amount of the volatile oils obtained from the bark were (9.4gm) it was 3.1% from the air dried bark, this percentage was good [13].

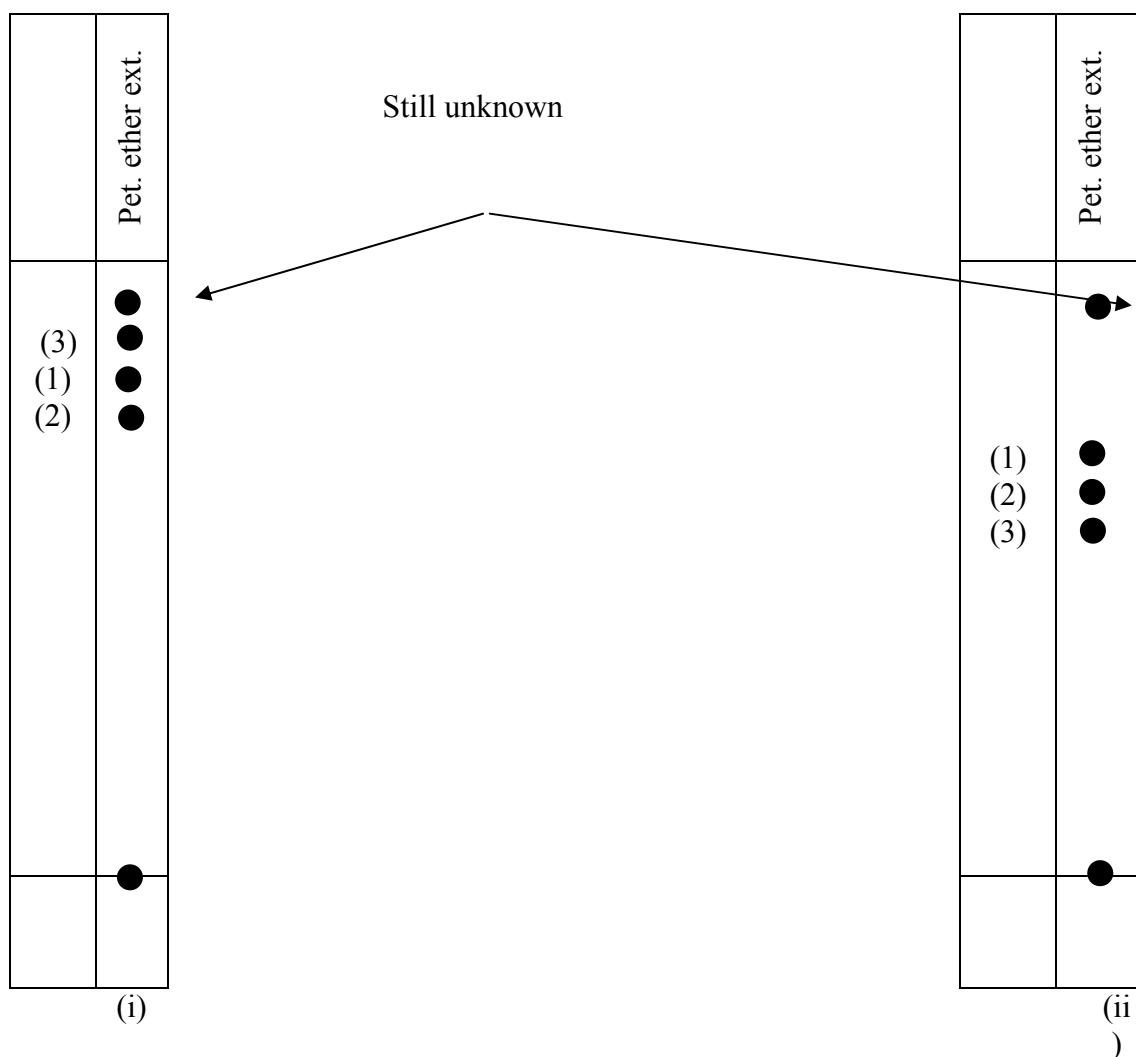
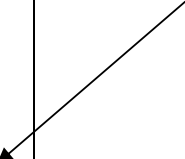


Fig. (1): Chromatogram of Some Volatile Oils (terpenes) presented in pet.ether extract, using solvent systems:

- (i)Hexane.
- (ii)Cyclohexane.

	Pet. ether ext.
(4)	● ● ●

Unknown Spot



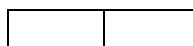


Fig. (2): Chromatogram of borneol as a volatile oil presented in the pet.ether extract using 5% ethyl acetate in benzene.

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