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Phytochemical Investigation of Crataegus monogyna (Part I)

Crataegus monogyna ile Fitokimyasal Tetkikler (Kısım I)

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As a result of a routine screen of Turkish plants for antitumor activity, the alcoholic extract of *Crataegus monogyna* (Rosaceae) showed activity (2.5 xl; 5.5 x O) toward the 9KB in vitro test system (1). This screening program was carried out by the Cancer Chemotherapy National Service Center, Bethesda, Md. USA. A survey of the literature revealed that many *Crataegus* species, including *C. monogyna*, have pharmacological activities such as cardioactive (2-6), vasodilator (7-12) and antibacterial (13).

An extensive chemical investigation has been performed with various *Crataegus* species and in *C. oxyacantha* crataegolic, ursolic and oleonolic acids (9, 14-19), crataegus lactone (6,20), saponins (21), choline and acetylcholine (22-24), vitexin-4-rhamnoside (19, 25-28), vitexin (26,27,29), quercetin (27,28) and some other compounds; in *C. survisepala* fifteen flavonoid compounds (30); in *C. pentagyna* saponins (31,32); in *C. submollis, C. rivularis carotens* (33); in *C. pinnatifida, C. chlorosarca, C. submollis* and in *C. douglasii* vitamin C. (33); in *C. lobulata, C. dippeliana, C. douglasii* hyperoside (34); in *C. hissarica, C. macracantha, C. lobulata* a rhamnoside (34); and finally in *C. monogyna* triterpenic acids (22,23), alkaloidal compounds, glycosides, vitamin C, red and yellow pigments (12,22,23,35,36), saponins (21), quercetin-3-rhamnogalactoside (37), acetylvitexin-4-rhamnoside (25), caffeic, chlorogenic acids (23), leucoanthocyanidin (38) have been found.

Although there are quite a number of papers published on *C. monogyna*, none of them studied the chemistry of the plant in detail.

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In this paper, the petroleum ether and benzene-chloroform soluble fractions of this plant were studied.

EXPERİMENTAL

The plant was collected from Belgrad forest in Istanbul in May 1967 and was identified as *C. monogyna* (Rosaceae) by Prof. Dr. A. Baytop (Univ. of Istanbul).

The dried powder of four kg of the stems and the leaves of the plant was macerated, then percolated with petroleum ether. Upon evaporation of the solvent under vacuum, about 20 g of a green residue was obtained (I). The marc was then extracted with 96 % ethanol, 276 g of a dark brown residue was obtained. This was dissolved in water and insoluble part was separated by centrifugation. The precipitate was extracted first with benzene, then with chloroform. Thin layer chromatography showed similar spots in both of the extracts, therefore they were combined (II) (60 g). Still an insoluble part was left from benzene and chloroform extraction, this was found to be soluble in alcohol (III) (94 g), water soluble part was evaporated to dryness. 120 g of a brown residue was obtained (IV). The plant was then extracted with 70 % aqueous ethanol, this yielded 44 g of a brown residue (V). In this investigation, fractions I and II were studied; fractions III, IV and V will be the subject of the second part of the phytochemical investigation of *C. monogyna*.

Fractionation of extract I: The green residue obtained from petroleum ether extraction was applied on a silicic acid-cellite (3:1) column (3x35 cm). The column was eluted with petroleum ether, benzene, chloroform and alcohol. Six compounds and an alkaloidal mixture were obtained.

Isolation and identification of n-triacontan ($C_{30}H_{62}$): Petroleum ether washings of the column yielded 2.1 g of a single pure compound. This was soluble in nonpolar solvents, slightly soluble in hot alcohol. It was crystallized from chloroform-alcohol (1:1) with a m.p. 63-64°C.; $[\alpha]$ $D^{20} \pm O°$ (in $CHCl_3$) UV spectrum gave no bands, IR spectrum showed characteristic saturated hydrocarbone bands at 2950, 2850, 1460 cm^{-1} (aliphatic C—H), 728 and 715 cm^{-1} (long chain —CH_2—). NMR spectrum gave two end methyl at 0.85 δ and 0.97 δ (3H, singlet for each), (—CH_2—)$_{28}$ at 1.25 δ ppm (56 H, singlet). Integration of the curve showed the presence of 62 hydrogens. Anal. Calc. for $C_{30}H_{62}$: C, 85.3; H, 14.69. Found C, 85.9; H, 14.75.

Unknown triterpenoid compound ($C_{30}H_{50}O_2$) : Petroleum ether-benzene (8:2) fractions gave 20 mg of a compound, upon crystallization from alcohol about 10 mg of crystals with a m.p. 247-250°C. were obtained. This gave a steroidal or a triterpenoid compound, the high melting point indicated that it could be a triterpenoid rather than a steroidal compound. IR spectrum showed the bands at 3450 cm^{-1} (OH), 2950 cm^{-1} (aliphatic C—H), 1740 cm^{-1} (carbonyl in a saturated six member ring). Anal. Calc. for $C_{30}H_{50}O_2$: C, 81.85; H, 11.31. Found C, 81.79; H, 11.66.

The amount of the compound was very little, therefore its structure could not be established.

Isolation and identification of nonacosanol-1 ($C_{29}H_{60}O$) : Petroleum ether-benzene (1:1) fractions yielded 500 mg of a compound with a m.p. 77-79°C. $[\alpha]$ $D^{20} \pm 0°$ (in $CHCl_3$). UV spectrum gave no bands. IR spectrum showed the bands of a saturated hydrocarbon alcohol at 3350 cm^{-1} (OH), 2950, 2850, 1470, 1380 cm^{-1} (aliphatic C—H), 1050 cm^{-1} (C—O), 720, 730 cm^{-1} (long chain —CH_2—). Since this spectrum indicates a hydrocarbon alcohol, N-bromosuccinimid test (39) was applied to find whether it was primary, secondary or tertiary alcohol. The test result showed a primary alcohol. NMR spectrum correlated this finding giving only one end methyl group at 0.9 δ (3H, singlet), $(—CH_2—)_{27}$ at 1.3 δ (54 H, singlet), OH group at 0.8 δ as it was seen by D_2O exchange and —CH_2—O group at 3.6 δ (2H multiplet). Integration of the curve showed 60 hydrogens. Anal. Calc. for $C_{29}H_{60}O$: C, 82.07; H, 14.15. Found C, 82.56; H, 14.35 %.

Isolation and identification of β-sitosterol ($C_{29}H_{50}O$) : This compound came together with nonacosanol-1 and with some other impurities from the later fractions of benzene-petroleum ether (8:2). Crystallization did not yield the pure compound, therefore it was purified by preparative thin layer chromatography. From 1 g of the mixture, 400 mg clean β-sitosterol was obtained, m.p. 135-136°C.; $[\alpha]$ D^{22} + 35° (in $CHCl_3$). UV gave a shoulder at 205 mμ (isolated double bond). IR spectrum showed the bands at 3450 cm^{-1} (OH), 2950, 2850, 1450, 1350 cm^{-1} (aliphatic C—H), the other bands were characteristic for steroidal compounds. IR spectra and R_f values comparison with the standard sample as well as the mixture melting point proved that this was β-sitosterol. Anal. Calc. for $C_{29}H_{50}O$: C, 84.05; H, 12.07. Found C, 84.17; H, 11.95.

Isolation and identification of ursolic acid ($C_{30}H_{48}O_3$) : This compound was obtained from benzene fractions together with a group of impurities and purified by preparative thin layer chromatography, m.p. 285-290°C. $[\alpha]$ $D^{20}+65$ (in pyridine), UV spectrum gave a band at 207 $m\mu$ (an isolated double bond). IR spectrum showed the bands at 3450 cm^{-1} (OH), a broad shoulder at 2500 cm^{-1} and carbonyl group at 1700 cm^{-1} indicated that this was an acid. IR spectra and R_f values comparison with the standard sample as well as the mixture melting point showed that this was ursolic acid. Anal. Calc. for $C_{30}H_{48}O_3$: C, 78.95; H, 10.52. Found C, 79.54; H, 10.31.

Isolation and identification of crataegolic acid ($C_{30}H_{48}O_4$) : Benzene-chloroform (8:2) washings gave 2.8 g of a rather impure fraction. After successive crystallizations from ethanol, about 80 mg of a pure compound was obtained, m.p. 269-271°C.; $[\alpha]$ $D^{22}+58$ (in pyridine). UV spectrum gave a band at 212 $m\mu$ (isolated double band). IR spectrum showed the bands at 3400 cm^{-1} (OH), a large shoulder at 2500 cm^{-1} and carbonyl at 1695 cm^{-1} indicated the presence of an acid. Methyl ester of this compound was prepared using a 30 % solution of diazomethane in ether. Melting point of the methyl ester was 226°C. [lit. 227-228°C. (40)]. IR spectra comparison with the standard sample showed that this was crataegolic acid. Anal. Calc. for $C_{30}H_{48}O_4$: C, 77.5; H, 10.09. Found C, 76.27; H, 10.17.

Alkaloid like compounds : The alcohol eluates of the column yielded a mixture of six compounds which gave orange spots with Dragendorff reagent. When this mixture was purified by a regular procedure for alkaloids, about 40 mg of an alkaloidal mixture was obtained. Thin layer chromatographic control using different solvent systems showed the presence of six compounds, they were not studied.

Fractionation of extract II : The benzene-chloroform combined extracts contained only two compounds different from that of extract I. These were separated by preparative thin layer chromatography.

Isolation and identification of caffeic acid ($C_9H_8O_4$) : About one gram of extract II was applied on twenty preparative cellulose plates (20 x 20 cm) as a streak and the plates were developed by 15 % aqueous acetic acid. The bright blue bands seen under UV light with an R_f value 0.4 were extracted with hot methanol. IR spectra and R_f values comparison with the standard sample proved that this was caffeic acid.

Isolation and identification of oleonolic acid ($C_{30}H_{48}O_3$) : Another one gram of extract II was applied on twenty preparative silica gel G plates (20 x 20 cm) as a streak and developed with chloroform-alcohol (9:1). The bands R_f 0.79 were extracted with chloroform. The compound thus obtained had a m.p. 305°C. The IR spectra and R_f values comparison on thin layer plates as well as the mixture melting point proved that this was oleonolic acid. Anal. Calc. for $C_{30}H_{48}O_3$: C, 78.95; H, 10.52. Found C, 78.47; H, 10.26 %.

SUMMARY

Petroleum ether and benzene-chloroform extracts of *Crataegus monogyna* yielded eight compounds and an alkaloidal mixture. These compounds were n-triacontan, nonacosanol-1, β-sitosterol, ursolic acid, crataegolic acid, an unknown triterpenic keto alcohol, caffeic and oleonolic acids. The alkaloidal mixture was not studied. Although the alcoholic extract of the plant showed antitumor activity toward 9KB in vitro test system, the activity shouldn't be in these fractions, but rather in the polar fractions.

ÖZET

Crataegus monogyna'nın petrol eteri ve benzen-kloroform ekstrelerinden bir alkaloidal karışım ve sekiz bileşik elde edildi. Bu maddeler n-triakontan, nonakosanol-1, β-sitosterol, ursolik asit, meçhul bir triterpenik keto alkol, krategolik asit, kaffeik asit ve oleonolik asittir. Elde edilen alkaloidal karışım incelenmemiştir. Bitkinin alkollü ekstresi 9KB in vitro test sisteminde antitümör aktivite göstermişse de, aktivite bu fraksiyonlardan ziyade polar fraksiyonlarda olmalıdır.

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Phytochemical Investigation of Crataegus monogyna (Part II)

Crataegus monogyna ile Fitokimyasal Tetkikler (Kısım II)

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Extracts III, IV and V obtained from *Crataegus monogyna* as described in a previous paper (1) were studied.

EXPERIMENTAL

Extract III:

When this extract was paper chromatographied and then controlled under UV light, blue streaks were observed; when the paper was sprayed with a 5 % solution of $FeCl_3$, blue-gray streaks were seen. These suggested the presence of leucoanthocyanidins.

A small amount of III was boiled with concentrated HCl, dark brown color turned into bright red, NaOH changed this color into blue. This test is characteristic for leucoanthocyanidins (2, p. 218).

Isolation and identification of leucocyanidin: To a solution of one gram of extract III in 50 ml of 96 % ethanol, 50 ml conc. HCl was added, the mixture was boiled about 20 minutes and cooled. The bright red solution was extracted with amyl alcohol, the latter was evaporated to a small volume under reduced pressure. The concentrated solution was applied on 20 sheets of Whatman no 1 paper (58 x 58 cm) as a streak and developed in a chamber using formic acid (98 %)-2N HCl (1:1). There was only one pink band with an R_f 0.27. This band was cut out and extracted with a mixture of MeOH-H_2O-Gl. AcOH (75:25:5). This extract was evaporated to a small volume under a vacuum, then applied on six sheets of Whatman no. 1 paper. The

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papers were developed using Gl.AcOH-conc.HCl-H_2O (30:3:10), again one pink band with an R_f 0.47 was obtained. The same procedure was repeated with a third solvent system namely n-BuOH-Gl.AcOH-H_2O (4:1:5:) again a single band was seen. The compound was extracted from paper strips as described above. Paper chromatographic comparison of this compound with a group of anthocyanidins showed that this compound was cyanidin. The UV spectrum in alcohol gave the maxima at 551 mμ and 278 mμ which were similar to that of cyanidin. In order to be sure that this compound was cyanidin, the alcoholic solution of it was evaporated almost to dryness in a platinum crucible, then fused with KOH. The phenolic and acidic parts of degradation product were separated as described in a previous paper (3). By comparison with standard acids and phenols using paper and thin layer chromatography, the acidic part was found to be protocatechuic acid and the phenolic part to be phloroglucin. These findings proved that this compound was cyanidin and the corresponding leucoanthocyanidin was leucocyanidin.

Extract IV:

Polyamide and cellulose thin layer chromatographic control of extract IV showed the presence of five flavonoid compounds. An attempt of separation of these five compounds on a polyamide column was unsuccesfull. About 1 g of IV as its alcoholic solution was applied on 30 sheets of Whatman no 1 paper (58 x 58 cm) as a streak and developed by using 15 % aqueous acetic acid. Five yellow bands were separated, these were cut out and extracted with 70 % aqueous alcohol, then evaporated to dryness.

Identification of the bands:

Quercetin: The first yellow band (R_f 0.02) when sprayed with a solution of lead acetate, showed an orange spot under UV light, indicating a possibility of a quercetin derivative. UV curve of this compound gave the characteristic bands of quercetin at 370 mμ and 257 mμ. The UV and IR spectra comparison with the standard sample proved that this was quercetin. Anal. Calc. for $C_{15}H_{10}O_7$: C, 59.61; H, 3.34. Found C, 59.62; H, 3.32.

Kaempferol: The second band (R_f 0.07) showed a yellow spot under UV light when sprayed with lead acetate, indicating the pre-

sence of a kaempferol derivative. UV spectrum of this compound gave the bands at 366 mμ and 265 mμ which were similar to that of kaempferol. The UV and IR spectra comparison with the standard sample proved that this was kaempferol. Anal. Calc. for $C_{15}H_{10}O_6$: C, 62.93; H, 3.49. Found C, 62.96; H, 3.50.

Rutin : The fourth band (R_f 0.48) with the above mentioned spraying was found to be a quercetin derivative. UV spectrum gave the bands at 360 mμ and 258 mμ. Comparison of the UV and IR spectra as well as the R_f values with that of the standard sample, this compound was found to be rutin. Anal. Calc. for $C_{27}H_{30}O_{16}$: C, 53.11; H, 4.91. Found C, 53.09; H, 4.92.

The third and the fifth bands were flavon glycosides, the glycon parts were found to be galactose in both of them, but the aglycons were not identified.

Extract V :

This extract showed the presence of two flavonoidal compounds on thin layer polyamide and cellulose plates. About 500 mg of extract V was applied on a polyamide column (4 x 4 cm), the column was eluted with aqueous methanol, the first fractions yielded about 60 mg of a yellow compound, the other fractions gave some impure compounds which were not studied.

Study of the yellow compound $C_{29}H_{32}O_{15}$: IR spectrum of this compound indicated the presence of an acetylflavon glycoside, by giving the bands at 3500, 2900, 1740, 1650, 1620, 1510, 1460, 1380, 1070, 1030, 915, 840, 760 and 690 cm^{-1} UV spectrum showed the maxima at 390 mμ and 260 mμ. The UV shifts with various reagents were studied, NaOAc produced no shift indicating an occupied C_7-OH group. A pronounced shift on band I (long wave band) was resulted by the addition of $AlCl_3$ indicating a free C_5-OH. No shift was obtained with NaOAc-H_3BO_3 showing the absence of a dihydroxy position (2, p. 116). Hydrolysis of the compound with 20 % HCl for 10 hours yielded glucose as the glycone part. This compound was quite similar to acetylvitexin rhamnoside which was given in literature (4) as $C_{29}H_{32}O_{15}$. The difference between the two compounds could be in their glycon parts. Since vitexin was not obtained as standard sample, the comparison was not possible, therefore its structure was not established.

SUMMARY

The alcohol, aqueous alcohol and water soluble fractions of *Crataegus monogyna* were studied and leucocyanidin,, quercetin, kaempferol, rutin were isolated and identified. Two other flavon glycosides were also isolated, their glycon parts were found to be galactose, but the aglycon parts were not identified. An acetyl derivative of a flavone glycoside was isolated, the findings suggested that it could be acetylvitexin glucoside.

ÖZET

Crataegus monogyna'nın alkol, sulu alkol ve suda çözünen fraksiyonları incelendi ve lökosiyanidin, kersetin, kamferol ve rutin izole ve teşhis edildi. Ayrıca elde edilen iki flavon glikozidinin şeker kısımları galaktoz olarak bulundu, fakat aglikon kısımları tespit edilemedi. Sulu kısımdan izole edilen asetilflavon glikozidin muhtelif bulgulara dayanarak asetilviteksin-4-glukozit olduğu tahmin edilmektedir.

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