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Citrus sinus peels as a source of polymethoxy flavonoids

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***Abstract*:**

Food processing waste as *Citrus sinus* peel is a type of agricultural waste that causes a great environmental problem. The problem could be solved by using this waste for production of valuable chemicals and pharmaceuticals. In this study three flavonoidal compounds were isolated from *Citrus sinus* peel. The isolated compounds were identified as nobeletin, sinensetin and isosinensetin using ^{13}C-NMR and ^{1}H-NMR techniques, in addition to melting point determination.. These compounds have various reported pharmacological activity.

Introduction:

Natural product contain different compounds that play an important role in drug discovery(1).Agriculture is the backbone of economic system of any country, but increased agricultural production associated with environmental problems as method of treating and disposing wastes adversely affect water, soil and air. Agriculturally related pollution attracts the scope of scientists. Number of reports and symposia developed to solve problem of agriculture waste to retard deterioration of environment quality. Food processing wastes are considered as agriculture production waste. Food processing waste has high volume and low strength and its discharge cause pollution problems as odor problem which is related to growth of microorganisms causing spread of diseases(2).Examples of food processing wastes (fruit wastes) include apple, pear, banana, citrus and citrus include mandarins, lemons, grapefruits and orange residues remaining after processing. About 70.7 million tons of orange produced annually. 30 million tons used in juice industry. Orange peels represent about 50% of wet fruit mass(3).The previous studies indicate the importance of all parts of fruit especially peels so we decided to examine compound present in orange peels. *Citrus sinus* is characterized by the presence of a wide range of diverse compounds such as flavonoids, chalcones, psoralens, coumarins, carotenoids, glycosides and essential oils.

Material and methods:

Plant material: *Citrus sinus* was collected from Egypt dried in shade for 1 month.

Chemicals: petrolium ether, methylene chloride and methanol (El-Nasr Company for Pharmaceutical Chemicals, Egypt) were of reagent grade, Analytical thin layer chromatography was performed on pre-coated aluminum sheets with silica gel 60 GF254 (20 x 20 cm x 0.2 mm thick). (Merck, Germany).Vanillin-sulfuric acid spray reagents used for visualization of TLC. Normal phase chromatography was carried out using silica gel G 60-230 mesh (Merck, Germany) packed by the wet method in the specified solvent.

Extraction procedure:

1.5 kilograms of the dried powdered orange peels were extracted by maceration in a percolator with MeOH (7x2L). The combined methanolic extracts were concentrated to a syrupy consistency under reduced pressure at 40 °C and then allowed to dry in a descicator over anhydrous CaCl2 to a constant weight (150 g, 10 %). The dried methanolic extract was dissolved in 150 mL MeOH, diluted with 450 mL distilled water. The produced solution is fractioned using petroleum ether and methylene chloride.

Methylene chloride fraction(4 g) was dissolved in small volume of methanol (4 mL) and then mixed well with about 2 g silica gel for column and left at room temperature to dry and applied onto the top of a silica gel packed glass column (50 x 4.5 cm, 300 g), previously packed in methylene chloride. Elution start with methylene chloride and developed by gradient elution using methylene chloride/methanol 100% (8 L), 99% (6.5 L), 97% (3.8 L), 95% (1.7 L), 90% (4.5 L), 85% (4.7 L), and finally washed with 100% methanol. Effluents, 100 mL fraction each, were separately concentrated, monitored by silica gel TLC plates in solvent system 3–15% v/v methylene chloride/methanol and the developed chromatoplates were heated after spraying with vanillin/sulfuric acid spray reagent. Similar fractions were pooled.

Fraction 1 obtained from column chromatography contain compound with R_f = 0.7 by TLC of silica gel GF254 using Methylene chloride: Methanol (97:3) as the developing solvent. This spot is separated as compound-1 (10 mg) by crystallization.

It was pale yellow needles with m. p. 134 °C - 137 ˚C.; ^{1}H-NMR (δ, DMSO-d_6): δH 6.868 (1H, s, H-3), 7.551 (1H, d, H-2'), 7.169 (1H, d, H-5'), 7.657 (1H,dd, H-6'), 3.858 (3H, s, H-3'), 4.030 (3H, s, H-4'), 3.885 (3H, s, H-5), 3.858 (3H, s, H-6), 3.980 (3H, s, H-7), 3.789 (3H, s, H-8); ^{13}C-NMR (δ, DMSO-d_6): δC 160 (C-2), 106.29 (C-3), 175.81(C-4), 147.14 (C-5), 143.5 (C-6), 150.93(C-7), 137.64 (C-8), 147.5 (C-9), 114.26(C-10), 119.32(C-1'), 108.91(C-2'), 149 (C-3'), 151.75(C-4'), 111.85(C-5'), 123.12(C-6'), 55.71(Methyl-3'), 55.67 (Methyl-4'), 61.14(Methyl-5), 61.39(Methyl-6), 61.88

(Methyl-7), 61.49 (Methyl-8).

Fraction 2 obtained from column chromatography contain compound with R_f = 0.36 by TLC of silica gel GF254 using Methylene chloride: Methanol (97:3) as the developing solvent. This spot is separated as compound-2 (15 mg) by crystallization.

It was pale yellow needles with m. p. 175 °C - 177 ˚C.; ^{1}H-NMR (δ, DMSO-d_6): δH 6.81 (1H, s, H-3), 7.23(1H, s, H-8), 7.56(1H, d, H-2'), 7.12(1H, d, H-5'), 7.67(1H,dd, H-6'), 3.9(3H, s, H-3'), 3.86(3H, s, H-4'), 3.78(3H, s, H-5), 3.81(3H, s, H-6), 3.97(3H, s, H-7); ^{13}C-NMR (δ, DMSO-d_6): δC 160.28(C-2), 106.38(C-3), 175.68(C-4), 153.92(C-5), 139.73(C-6), 157.4(C-7), 97.35(C-8), 149(C-9), 112.03(C-10), 123.13(C-1'), 109.15(C-2'), 151.54(C-3'), 151.66(C-4'), 111.64(C-5'), 119.43(C-6'), 55.68(Methyl-3'), 55.86(Methyl-4'), 61.82(Methyl-5), 60.97(Methyl-6), 56.45(Methyl-7).

Fraction 3 fractions (126-139) eluted with 99% methylene chloride/ methanol , showed three spots at R_f values 0.16 (band 1), 0.2 (band 2) and 0.24(band 3) on silica gel TLC plate developed in methylene chloride – methanol (97:3 v/v). Band 2 and band 3, showed quenching under UV lamp but acquires a yellow color after heating with vanillin/sulfuric acid spray reagent. Group 1 was dissolved in a minimum amount of ethyl acetate (2 mL), mixed with about 1 g silica gel, dried and loaded onto the top of a silica gel glass column (35 x 1 i. d., 20 g) previously packed in petroleum ether. Gradient elution (Table 1) is adopted with different proportions of petroleum ether in ethyl acetate {50% (100 mL), 40% (100 mL), 30% (100 mL) and 20% (100 mL)}. The effluents, 25 mL fractions, were monitored by TLC on silica gel plates, and similar fractions were pooled. Subgroup a, fraction 14, was further purified by re-chromatography on silica gel column (35 x 1 i. d, 20 g) previously packed in methylene chloride. Elution started with methylene chloride (100 mL), then using methylene chloride / methanol {99% (550 mL), 97% (390 mL) and 95% (190 mL) }. The effluents, 10 mL fractions, were monitored by TLC on silica gel plates, and similar fractions were pooled. Compound-3 (9 mg) was obtained from fractions 75-85, and was shown as one spot on TLC silica plate.

Table (1): Results of column chromatography of group 3 (fraction No. 126-139).

Solvent system (petroleum ether /ethyl acetate)	Fractions	Fraction collected
50 :50	1-4	Subgroup a (14)
60:40	5-8	
70:30	9-12	
80:20	12-16	

Compound-3 was isolated as colorless needles (9 mg), m.p. 197 °C- 199 ˚C.; 1H-NMR (δ, DMSO-d6): δH 6.69 (1H, s, H-3), 6.66 (1H, s, H-6), 7.56(1H, d, H-2'), 7.13(1H, d, H-5'), 7.66(1H,dd, H-6'), 3.94(3H, s, H-3'), 3.92(3H, s, H-4'), 3.96(3H, s, H-5), 4.04(3H, s, H-7), 3.94(3H, s, H-8); 13C-NMR (δ, DMSO-d6): δC 162.9(C-2), 107.09(C-3), 180.29(C-4), 157.82(C-5), 94.32(C-6), 158.98(C-7), 131.84(C-8), 153.98(C-9), 110.40(C-10), 124.94(C-1'), 109.07(C-2'), 150.99(C-3'), 153.13(C-4'), 112.94(C-5'), 121.22(C-6'), 56.6 (Methyl-3'), 56.6(Methyl-4'), 57.06(Methyl-5), 56.7(Methyl-7), 61.98(Methyl-8).

Result and discussion:

The hydro - alcoholic extract of *Citrus sinus* was farctioned with petroleum ether, methylene chloride and ethylacetate. The methylene chloride fraction was purified and three compounds were obtained. By means of spectroscopic analysis, they were characterized as nobiletin [(4)], sinensetin [(5)] and 5,7,8,3',4'-pentamethoxyflavone [(6)].

Compound-1(Figure-1) was isolated as pale yellow needles (10 mg), m. p. 134 °C- 137 ˚C, soluble in methylene chloride. It produces florescence under UV lamp λ254 and acquires a yellow color after spraying with vanillin/sulfuric acid reagent and heating at 110˚C for 1 minute. Chromatographic analysis on silica gel TLC plates GF254 and 97% methylene chloride/methanol showed Rf value of 0.7.

^{13}C spectrum of compound 1 revealed the presence of twenty one signals. The most indicative signals are carbon at δC 175.81 confirming the presence of a carbonyl group, the olefinic carbons at δC 106.29 - 160.26 and the rest of the carbons at the range from 55.67 - 61.88 ppm reflecting their aliphatic nature. From the above data, compound-1 contain methyl-oxy group.

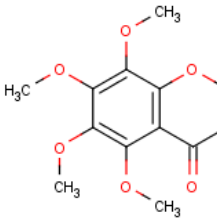
Compound -2 (Figure-2) was isolated as pale yellow needles (15 mg), m. p. 175 °C- 177˚C, soluble in methylene chloride.

It produces florescence under UV lamp λ254 and acquires a yellow color after spraying with vanillin/sulfuric acid reagent and heating at 110˚C for 1 minute. Chromatographic analysis on silica gel TLC plates GF254 and 97% methylene chloride/methanol showed R_f value of 0.36

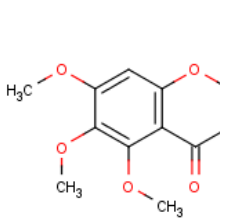
^{13} C spectrum of compound-2 revealed the presence of twenty one signals. The most indicative signals are carbon at δC 175.68 confirming the presence of a carbonyl group, the olefinic carbons at δC 106.38 - 160.28 and the rest of the carbons at the range from 55.68 - 61.82 ppm reflecting their aliphatic nature. From the above data, Compound-2 contain methyl-oxy group.

Compound-3(Figure-3) was isolated as colorless needles (9 mg), m. p. 197 °C- 199 ˚C, soluble in methylene chloride. It produces florescence under UV lamp λ254 and acquires a yellow color after spraying with vanillin/sulfuric acid reagent and heating at 110˚C for 1 minute. Chromatographic analysis on silica gel TLC plates GF254 and 97% methylene chloride/methanol showed R_f value of 0.2.

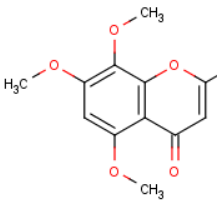
^{13} C spectrum of compound-3 revealed the presence of twenty one signals. The most indicative signals are carbon at δC 180.29 confirming the presence of a carbonyl group, the olefinic carbons at δC 94.32 – 162.9 and the rest of the carbons at the range from 56.6 - 61.98 ppm reflecting their aliphatic nature. From the above data, Compound-3 contain methyl-oxy group.



(Figure-1)



(Figure-2)



(Figure-3)

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