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A CONTRIBUTION TO THE CHEMISTRY OF STERCULIA DIVERSIFOLIA DONN CULTIVATED IN EGYPT

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From Sterculia diversifolia Donn, luteolin, quercetin, kaempferol, quercetin-3- arabinoside and P-Coumaric and isochlorogenic acids were isolated from the leaves. While, caffeine, theobromine, theophylline and choline were isolated from the defatted seeds. Lipids of all the organs showed the presence of a hydrocarbon m.p. 58°C, α-amyrin and B-Sitosterol in the unsaponifiable fraction. Fatty acids were found to be oleic, myristis, palmitic and stearic.

Sterculia diversifolia Donn (Brachychiton populneum R.Br., Kurajong, Bottle tree) is a common tree growing in Australia and cultivated in Egypt and Known as poodret El-Afrit بودرة العفريت. The plant belongs to Family Sterculiaceae and the genus has many medicinal uses[1-5]. In previous papers the macro and micromorphological characters of the plant were reported[6,7]. Concerning the chemistry of Sterculia diversifolia Donn, little is published[8] and nothing about that cultivated in Egypt. Preliminary Phytochemical screening showed the presence of flavonoids in the leaves and traces in the stems and aerial branches,

alkaloids and basic nitrogenous substances in the seeds, lipoidal matter and unsaturated sterols and/or triterpenes in all the investigated organs. Such lack of information about the chemistry of the plant and the presence of many different components, as indicated by screening, encouraged the present investigation.

EXPERIMENTAL

Plant Material. The plant was collected from public and private gardens in Assiut and identified by the late Prof. Dr. F. Y. Amin, Prof. of Horticulture, Faculty of Agriculture, Assiut University . The plant organs were separated, air-dried and powdered to No. 40 powder.

Extraction, Separation and Indentification:

A. The Flavonoids:

3Kg powdered leaves were defatted with pet-ether(60-80°C) and extracted with ethanol (70%). The aqueous mother liquor remaining after concentration of the extract, was extracted with ether, EtOAc and n-butanol successively. TLC (Polyamide and MeOH or MeOH-gl. acetic acid-H_2O (18-1-1) of EtOAc and n-butanol fractions revealed a relatively similar picture. Crystalline yellow needles were separated from the concentrated ethereal fraction, filtered, then washed thoroughly with ether (F_1). The concentrated filtrate was chromatographed over

polyamide column and compounds F_2, $F_2 + F_3$, F_4 and F_5 were eluted with 5%, 10%, 20% and 40% MeOH in H_2O respectively. Mixture containing $F_2 + F_3$ was fractionated over preparative silicagel (E. Merck) and toluene-EtOAc[5-4]. Column-chromatography of the concentrated EtOAc fraction over silicagel separated compounds G_1 ($CHCl_3$ - EtOAc 3:2) and G_2 (MeOH - EtOAc 1:9).

Compound F_1:

identified as luteolin[9], m.p. 333-5°C, UV λ_{max}^{MeOH} (nm): 256, 270, 350; + NaOAc, + 40 nm; + NaOAc/H_3BO_3, + 30 nm; + NaOMe, + 55 nm; + $AlCl_3$, + 68 nm; + $AlCl_3$/HCl, + 10 nm; M. m.p. and Co-chromatography confirmed the identity.

Compound F_2 :

identified as P-coumaric acid[10], m.p. 198-200°C, UV λ_{max}^{MeOH} (nm): 215, 225 (Sh), 239, 316 (inf). R_f: PC, Whatman No. 1, n-butanol-n-butylacetate-acetic acid-H_2O (9 - 28 - 47 - 61) (descending) 0.84; TLC, silicagel, toluene-ethylformate-formic acid (5-4-1) 0.14. M.m.p. and Co-Chromatography confirmed the identity.

Compound F_3:

Non-identified (too-little material) UV λ_{max}^{MeOH} (nm): 256 (Sh), 263, 266, 272, 400; + NaOAc, — ; + NaOAc/H_3BO_3, — ; + NaOMe, — ; + $AlCl_3$, + 15 nm; + $AlCl_3$/HCl, + 14 nm. R_f: PC, 15% acetic acid, 0.7; TlC, polyamide, benzene-butanone - MeOH (4 - 3 - 3), 0.08, Silicagel, toluene - $CHCl_3$-acetone (40 - 25 - 35), 0.38.

Compound F_4:

identified as kaempferol[9]; m.p. 227-280°C, UV λ_{max}^{MeOH} (nm): 276, 367; + NaOAc, + 13 nm; + NaOAc/H_3BO_3, — ; + NaOMe, + 43 nm; + $AlCl_3$, + 161 nm; + $AlCl_3$/ HCl, + 61 nm. M.m.p. and Co-Chromatography confirmed the identity.

Compound F_5 :

identified as quercetin[9], m.p. 316 -18°C; UV λ_{max}^{MeOH} (nm): 255 , 330 (Sh), 370; + NaOAc, + 51 nm; + NaOAc/H_3BO_3, + 27 nm; + NaOMe, + 34 nm; + $AlCl_3$, + 57 nm; + $AlCl_3$/HCl, + 55 nm. M.m.p. and Co-chromatography confirmed the identity.

Compound G_1:

identified as isochlorogenic acid[10] m.p. 169°C, UV λ_{max}^{MeOH} (nm): 218, 236 (Sh), 330. R_f: PC, n-butanol-n-butylacetate-acetic acid-H_2O(9 - 28 - 47 - 16) (descending) 0.04; TLC, silicagel, EtOAc - toluene - acetic acid (5 - 4 - 2) 0.57. M.m.p. and Co-chromatography confirmed the identity.

Compound G_2:

identified as quercetin -3-arabinoside[9], m.p. 210°C, UV λ_{max}^{MeOH} (nm): 260, 360; + NaOAc, + 40 nm; NaOAc/H_3BO_3, + 25 nm; + NaOMe, + 40 nm; + $AlCl_3$, + 70 nm; + $AlCl_3$/ HCl, + 40 nm. R_f: TLC, silicagel, EtOAc - formic acid - H_2O (10 - 2 - 3) 0.59; polyamide, MeOH, 0.31; PC, 15% acetic acid, 0.7. Acid hydrolysis: quercetin identified by UV analysis and Co-chromatography, arabinose identified by TLC.

B. The alkaloids:

10 kg of the dried powdered seeds were defatted with pet.-ether (60-80°C) then extracted with ethanol (70%) containing 5% tartaric acid. The aqueous mother liquor was

extracted with $CHCl_3$ and the residue of the $CHCl_3$ extract was chromatographed over silicagel column. Compound K_1, K_2 and K_3 were eluted successively by acetone. Compound K_1 was purified by repeated concentration, refrigeration and filtration then descarding the residue. The remaining solution was dried and dissolved in warm water followed by concentration and refrigeration. Few white plates were obtained which were filtered and dried over anhydrous calcium chloride. Compound K_2 was purified by preparative TLC, silicagel, $CHCl_3$ (saturated with ammonia) - acetone-isopropanol -n- propanol (16 - 2 - 1 - 1), flourescent band (UV) was eluted with ethanol (95%) dried under reduced pressure, dissolved in $CHCl_3$ and refrigerated. Needle-shaped crystals were precipitated, filtered and dried. Compound K_3 was recrystallised from water after refrigeration.

The aqueous mother liquor remained after extraction of the alkaloids with $CHCl_3$ was concentrated and chromatographed over alumina column. Upon elution with EtOH , fractions 9-22 were collected and dried (K_4) . Reineckate salt was prepared by the usual method.

Compound K_1:

identified as theophylline[11], m.p. 270 - 5°C. R_f: TLC, silicagel, EtOAc - MeOH - acetic acid (8 - 1 -1)0.45, $CHCl_3$- formic acid - EtOH (88 - 2 - 10) 0.5, benzene - acetone (8-7) 0.6; visualizing agent was Dragendorff reagent followed by 1% $AgNO_3$ in 5% H_2SO_4 [12] the spot of alkaloids attains red colour. Positive murexide test. M.m.p. and Co-chromatography confirmed the identity.

Compound K_2:

identified as caffeine[11] m.p. 237 - 9°C (sublimation).

R_f 0.41, 0.57, 0.07. Positive Murexide test. Microcrystallisation: small rods with gold chloride, long needles with mercuric chloride. UV $\lambda_{max}^{H_2O}$ (nm): 272. nmr: 2.40 (s), 5.98 (s), 6.42 (s), 6.60 (s) ppm. M.m.p., Co-chromatography and the superimposable IR Spectra confirmed the identity.

Compound K_3:

identified as theobromine, [11], m.p. 356 - 8°C (sublimation). R_f, 0.36, 0.34, 0.41. Positive murexide test. Microcrystallisation: unstable hair - like needles with goldbromide small rods and plates with platinic chloride UV $\lambda_{max}^{H_2O}$ (nm): 273. M.m.p., Co-chromatography and inseparable IR spectra confirmed the identity.

Compound K_4:

identified as choline. R_f: TlC, alkaline alumina , $CHCl_3$ - EtOH (1-1), 0.78, violet colour with modified Dragendorff's reagent. Reineckate, m.p. 373°C. M.m.p. , Co-chromatography and inseparable IR Spectra confirmed the identity .

C. The lipids:

1- Fatty matter from the leaves:

1 kg. air-dried powdered leaves was extracted with Pet. ether (60-80°C) and concentrated where a dark green, Sticky and resinous residue was abtained (15 g.) 10 g of this residue was saponified and the unsaponifiable fraction was chromatographed over alumina column. Compounds A,B and C were eluted with benzene and crystallised from MeOH.

2- Fixed oil from the seeds:

The oil was extracted with pet.-ether (60-80°C) and dried over anhydrous sodium sulphate (20% yield). The unsaponifiable fraction revealed the presence of substances A,B and C. The fatty acids of the saponifiable part were methylated after extraction with ether and analysed by GLC. These fatty acids are: oleic [illegible]%, palmitic 13.3%, stearic 7.5% and traces of myristic.

Fixed oil of the seeds is orange-yellow in day light, greenish-yellow in UVL, agreeable oily taste, faint characteristic odour, sp.gr.=0.903, Iodine value = 95, saponification value = 185, fatty acids 52%, unsaponifiable matter 6.5% w/w.

Compound A = wax, m.p. 58°C, negative liebermann-Burchard.

Compound B:

identified as α-amyrin, m.p. 185°C M.m.p., Co-Chromatography and inseperable IR spectra confirmed the identity.

Compound C:

identified as B-sitosterol, m.p. 136°C, acetate m.p. 128°C, chromatography over reactive layers ($AgNo_3$-impregnated silicagel, pet-ether (60-80°C)-$CHCl_3$-gl. acetic acid(75-25-0.5), one spot identical to B-sitosterol acetate. M.m.p., Co-chromatography and inseperable IR spectra confirmed the identity.

A contribution to the chemistry of Sterculia diversifolia Donn cultivated in Egypt

Quantification of the alkaloids:

It is evident from the course of isolation of alkaloids that caffeine is the major one, theobromine is moderate and theophylline is minor (approximately 0.1 mg in Kg). Consequently, caffeine and theobromine were determined quantitavely and theophylline was discarded.

The method of determination is based on the measurement of the absorbance of caffeine at 272 nm and of theobromine at 273 nm. Standard calibration curves were prepared from serial dilutions of pure caffeine in distilled water and theobromine in N/10 sodium hydroxide and obeyed Bear's law in concentrations of 1 to 10 mg/ml.

Four samples, each weighed 50 g. of powdered seeds were defatted and extracted with EtOH (70%). The residue, remained after removal of the solvent, was subjected to preparative PC using whatmann no 1 and n-butanol: ammonia : water (100:2:16) Bands having R_f 0.25 and 0.72(UVL.) were separated, eluted with absolute ethanol, dried and residue dissolved in 100 ml distilled water in case of caffeine and N/10 NaOH in case of theobromine.

Each Kg of the seeds contains 100 mg of caffeine and 20 mg of theobromine.

DISCUSSION

The investigation of the different organs of Sterculia diversifolia Donn cultivated at Assiut, Egypt revealed the presence of flavonoids in the leaves and purine bases or alkaloids together with choline in the seeds. The lipid obtained from the latter was saponified and the saponifiable and unsaponifiable fractions were studied.

The isolation of the flavonoidal components, kaempferol, luteoline, quercetin and its 3-arabinoside, in addition to isochlorogenic and P-coumaric acids constitutes the first report of their presence in the genus Sterculia [13-16]. On the other hand, the reported glucuronides of apigenin, luteolin, scutallerein and 6-hydroxyluteolin are absent [14-15].

According to Raffouf[11], some genera of the Family Sterculiaceae contain purine bases and the genus Sterculia is one of them. Fraise[17] reported the presence of caffeine only in some Sterculia species. Other reports[18,19] indicate the presence of caffeine or components that give a positive meruxide test. The presence of caffeine, theobromine and theophyline in Sterculia diversifolia Donn seeds put the plant in a unique situation among the genus.

Plants of the order Malvales, to which the family Sterculiaceae belongs, are known to contain cyclopropene fatty acids (sterculic and malvalic) as components of their lipids[20,21]. These acids could not be traced during our investigation upon the oil in hand. The labile nature of them as they easily decompose or undergo polymerisation even at room temperature[21,22] and the presence of the oleic acid in a high percent (79) in relation to them (about 10) might be the causes of the negative results. However, biological studies of the oil[23] indicated the presence of a cyclopropene neucleus. The symptoms noticed on the birds fed the oil or the seeds are similar to those reported in the literature[8,22,24]. In addition the isolation of one of them (malvalic) and the positive Halphen' stest proove its presence.

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الدراسة الكيمائية لنبات الستركبولياديفرسيفوليا دون المنزرع فى مصر

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نبات الستركيوليا ديفرسيفوليا دون المعروف فى مصر تحت اسم بودرة العفريت يتبع العائلة الاستركيولية وموطنه الاصلى استراليا وقد تمت زراعته حديثا فى مصر . ولهذه العائلة عدة احناس تابعة لها الكثير من الاستعمالات الطبية وكذلك تم فصل العديد من المركبات الفعالة منها .

وفى هذه الدراسة تم فصل مواد فلافونيدية هى الليوتيولين والكورسيتين والكمفرول والكورسيتين ـ٣ـ ارابنيوسيد وكذلك حمض الباراكوماريك والايزوكلوروجنك من الاوراق ، ومن البذور تم فصل اشباه فلويدات هى الكافيين ،شيوفوللين وثيوبرومين وكذلك قاعدة نتروجينيه رباعية هى الكولين أما المواد الدهنية من اعضاء النبات المختلفة فوجد انها تحتوى على احماض الاوليك والميرستيك والبالمتيك والاستياريك فى الجزء المتصبن ومن ناحية اخرى وجد ان الجزء الغير متصبن يحوى مادة شمعية وبيتاسيتوستيرول والفا اميرين وقد تم فصل زيت بنسبة ٢٠٪ من البذور وقد درست خواصه . وقد تم تعيين نسب كل من الكافيين والثيوبرومين ووجد أن الكيلو جرام من البذور يحوى ١٠٠ مجم من الأول و ٢٠ مجم من الثانى . أما الثيوفيللين فقد تم فصله بكمية ضئيلة (ار. مجم تقريبا) .

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