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A PHYTOCHEMICAL STUDY OF THE FRUITS OF CERTAIN CASSIA SPECIES CULTIVATED IN EGYPT

Samia M. El-Sayyad & Hanaa M. Sayed
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

ABSTRACT

The friuts of Cassia javanica Linn., C. siamea Lam., C. fistula L. and C.didymobotrya Fres. are subjected to a phytochemical screening followed by isolation and identification of their constituents. The study revealed the presence of flavonoids, anthraquinones, chromones, alkaloids, sterols and/or triterpenes together with hydrocarbons and alcohols in the different fruit pericarps.

The physical as well as chemical characteristics of the fixed oil of the seeds are determined. In addition the fatty acids of each individual seed oil are identified by gas-liquid chromatography.

INTRODUCTION

Cassia species are commonly used in folkloric medicine as purgatives and also for treatment of joints, spleen, liver disorders and leprosy[1,2] . It was mentioned that the fruits and heart-wood of C.siamea Lam. have been used as dyes[3]. C. fistula L. has purgative activity similar to senna[1] in addition to its effect against malaria, blackwater fever, blood poisoning, anthrax and dysenteries[3]. C.didymobotrya Fres. has

also purgative and antimalarial effects[3].

About seventeen Cassia species are cultivated and grown in Egypt[4]. From them C.javanica Linn., C. siamea Lam., C. fistula L. and C.didymobotrya Fres. were chosen to perform the present study on their friuts, which are reputed for their importance in public medicine[3].

The trees (C. javanica Linn., C. siamea Lam., C.fistula L.) and the shrub(C. didymobotrya Fres.) are cultivated as ornaments in public and experimental gardens.

A perusal of the literature revealed that several reports appeared on the first three species abroad[5-20]. Chrysophanol, rhein and aloe-emodin were isolated from the leaves of C. javanica Linn. and C. fistula L., in addition to 1,8-dihydroxy anthraquinone, sennosides A & B from the latter. Bianthraquinone glycoside and kaempferol were isolated from C. fistula L. flowers. Three pigments cassiamin A,B & C were isolated from the roots of C.siamea Lam., and the alkaloid siamine from its seeds.

Moreover, certain studies were performed on other parts of the plants[21-28]. From C. javanica Linn. leaves, ∝-and B-amyrin, kaempferol, kaempferol-3-methyl ether, kaempferol 7-methyl ether and quercetin, also rhein, emodin, sinapic acid and leucoanthocyanin were isolated and identified. New isoquinolone alkaloids were isolated from C.siamea Lam. leaves. Chrysophanol, rhein, aloe-emodin, rhein-rhammnoside and sennosides were isolated from the leaves of C.fistula L. From C.didymobotrya Fres. leaves, chrysophanol, aloe-emodin, kaempferol 3-rhammnoside, isoquercitrin and didymobotrine alkaloid were isolated.

This investigation deals with the fruits which received little attention in the previous studies.

RESULTS AND DISCUSSION

The oils prepared from the seeds of each individual fruit occur as clear liquids, freely soluble in light petroleum, ether and chloroform, insoluble in ethanol. The physical and chemical characteristics are presented in Table 1. The high acid values of the oils indicated the presence of certain amounts of free acids, so these oils are not suitable for human consumption (edible oils should not contain more than 1%& free acids). In addition the iodine values of the oils are almost similar to those of the semi-drying oils. The determined percentages of the unsaponifiable matter revealed that the oils contain small amounts of waxes and higher hydrocarbons (edible oils with unsaponifiable matter not more than 1-2%).

The fatty acids of the seed oils obtained after saponification were esterified and studied by gas-liquid chromatography and the results are shown in Table 2.

The percentages of the unsaturated fatty acids amounting to 58.76%, 70.7% & 53.7% in C.javanica Linn., C. siamea Lam.,and C. didymobotrya Fres. respectively present a further indication of the semi-drying nature of these oils.

The percentage of unsaturated fatty acids of C.fistula L. as represented by palmitic, arachidonic, myristoleic and oleic acids, in a total percentage of 33.93% is lower than that of the other species.

The unsaponifiable fractions of the petroleum-ether extracts of the different pericarps were separated into their components by column chromatography followed by preparative thin-layer chromatography or recrystallisation and the identified compounds are presented in Table 3.

The ethanolic extract of each pericarp was fractionated between ether and ethyl acetate. Each subsequent fraction was separated into its components by column chromatography. The ethereal fractions were found to contain flavonoids and anthraquinones while ethyl acetate contained in addition alkaloids and chromones.

Alkaloids were detected only in C.siamea Lam. and C.didymobotrya Fres. pericarps. From the former siaminine B was isolated as its hydrochloride salt, identical to that of the leaves[26]. Moreover, didymobotrine previously reported in C. didymobotrya Fres. leaves[25], was also isolated from the pericarp. Choline base was detected only in C. didymobotrya Fres. pericarp.

A leuco-anthocyanin was obtained from the pericarp of C. javanica Linn. only. In addition, traces of anthraquinone derivatives were isolated but not fully identified.

A chromone (cassiachromone) was isolated from the pericarp of C.siamea Lam. only, which is identical to that previously found in the leaves[23]. Table 3 shows a pilot presentation of the isolated constituents of the different pericarps.

EXPERIMENTAL

Plant Material:

The fruits of C.javanica Linn. were obtained from the Botanic Island at Aswan, those of C.siamea Lam. from Zohria Gardens at Cairo, while C. didymobotrya Fres. and C.fistula L. were collected from the Horticulture Experimental Garden,

Faculty of Agriculture in Assiut. All were identified by the Late professor Dr. F.Y. Amin, Prof. of Floriculture and Horticulture, Faculty of Agriclture, Assiut University.The fruits were collected during winter 1981. The pericarps and seeds were separated, dried, powdered to sieve No. 40 and kept in well-closed dark containers.

Extraction and Separation:

Fifty g. of each powdered separated seeds were extracted with petroleum-ether (60-80°C). The collected extracts were washed with aqueous sodium hydroxide (2-3%), then with alcohol-water mixture (1:1) several times till free from alkali, then allowed to stand overnight with charcoal. Each extract was dehydrated over anhydrous sodium sulphate, completely freed from the solvent and the obtained oils were heated at 100°C for five hours, then allowed to cool at room temperature.

The physical as well as the chemical characteristics of the prepared fixed oils were determined according to the official methods of analysis of the Association of Official Analytical Chemists[29].

The individual seed oils were saponified and fractionated with ether. The methyl esters of the fatty acids were prepared by the usual method[30] and examined by gas-liquid chromatography.

Half kg. of each powdered pericarp was extracted separately with petroleum-ether (60-80°C) followed by ethanol (70%). The concentratrated ethanolic extracts were subjected to solvent fractionation using ether and ethyl acetate. The individual extracts were concentrated. The petroleum-ether extract was

saponified and fractionated with ether. The unsaponifiable matters were chromatographed over alumina (Prolabo) columns and eluted with solvents of increasing polarities. The subsequent fractions containing the hydrocarbons and long chain alcohols were separated and purified over preparative silica gel G plates, slurried with 10% acetic acid and developed by benzene-ethyl acetate (1:1). The obtained products were crystallised, dried and identified.

Ether and ethyl acetate fractions were chromatographed over silica gel (E. Merck) columns and eluted with solvents of increasing polarities; more purification of the products was done on preparative silica gel G plates, with ethyl acetate methanol-water (100:16:14) (system i) or which toluene ethyl formate-formic acid (5:4:1) (system ii)or with cellulose plates and chloroform-acetic acid-water(50:45:5) (system iii).

Components of the Unsaponifiable Matters:

Heptacosane: White micro needles (EtOAC), m.p. 59°C,IR(KBr), 2920, 2840, 1470, 1370 and 725 cm^{-1}, Co-chromatography and m.m.p. with authentic sample confirm its identity[6].

Hentriacontane: White crystals (EtOAC), m.p. 68°C, IR(KBr), 2910, 2820, 1450, 1360 and 730 cm^{-1}, identified by Co-chromatography and m.m.p. with authentic sample[6].

Cerotic Acid: Colourless micro-needles(Acetone),m.p. 82-84°C, IR(KBr) 3230, 2300, 1450, 1330, 1700 and 720 cm^{-1}, identified by Co-chromatography and m.m.p. with authentic sample[6].

Unidentified Straight-Chain Primary Alcohol: Colourless flakes (EtOAC), m.p. 67-69°C. IR(KBr), 3450, 2950, 2830,1465 1065 and 780 cm^{-1}, lack of material prevented further identi-

fication.

β-Sitosterol: White flakes (MeOH), m.p. 136-37°C, acetate m.p. 125-27°C, identification was confirmed by superimposable IR spectra, m.m.p. and Co-chromatography with authentic sample.

α-Amyrin: White micro needles (MeOH), m.p. 184-86°C, acetate m.p. 226-28°C, identified by m.m.p., superimposable IR spectra and Co-chromatography with authentic sample.

β-Amyrin: White fine needles (MeOH), m.p. 200-202°C, acetate m.p. 202-204°C, identified by m.m.p., superimposable IR spectra and Co-chromatography with authentic sample.

Unidentified Sterol: White micro-needles, m.p. 180-82°C ,IR (KBr), 3400, 1370, 1275, 1080, 1060 and 760 cm^{-1}, positive reaction for sterols(green colour with Lieberman Burchard test); lack of material prevented further identification.

Anthraquinones and Falvonoids:

Chrysophanol: Red residue (Acetone), m.p. 197-8°C, UV λ_{max}^{EtOH} 225, 257, 229, 288, 433 nm, IR (KBr) 1680, 1632 & 1611 cm^{-1}, identified by comparison with published data [5,31], m.m.p. and Co-chromatography with authentic sample.

Aloe-emodin: Orange-red residue (Ether), m.p. 230°C, UV λ_{max}^{EtOH} 225, 256, 278, 287, 425, 457 nm, IR(KBr) 3400, 1673, 1629 & 1610 cm^{-1}, identified by comparison with published data [5,31], m.m.p. and Co-chromatography with authentic sample.

Emodin : Red residue (Acetone), m.p. 256-9°C, UV λ_{max}^{EtOH} 223, 253, 266, 289, 438 nm, IR (KBr) 3390, 1675, 1631 & 1610 cm^{-1}, identified by comparison with published data [5,31], m.m.p. and Co-chromatography with authentic sample.

Physcion: Pinkish-red residue (Acetone), m.p. 210°C, UV λ_{max}^{EtOH} 225, 255, 266, 288, 436, nm. IR (KBr) 1678, 1623 & 1610 cm^{-1}, identified by comparison with published data [5,31], m.m.p. and **co-chromatography with anthentic sample.**

Rhein: Orange-red residue (Acetone), m.p. 330°C UV λ_{max}^{EtOH} 228, 258, 431 nm, IR (KBr) 3400, 1700, 1670 & 1628 cm^{-1}, identified by comparison with published data [5,31], m.m.p. and Co-chromatography with authentic sample.

Rhein glycoside: Yellowish-brown amorphous residue, m.p. 284°C (sublimation), UV λ_{max}^{EtOH}, 230, 270, 285 (sh), 239 nm; soluble in water acetone, ethanol, insoluble in organic solvents. Acid hydrolysis yielded rhein (identified by UV data and Co-chromatography with authentic sample), rhamnose and arabinose.

Unidentified anthraquinone: Dark reddish-violet($CHCl_3$), m.p. 245°C (decomposition) UV λ_{max}^{EtOH}, 280, 295 (sh), 515 nm, positive reaction for anthraquinones (Borntragers reaction[5]); lack of material prevented further identification.

Kaempferol: Yellow residue (MeOH), m.p. 282-84°C, UV λ_{max}^{MeOH}; 268, 370 nm, + NaOAC: 274, 384 nm, + NaOAC/H_3BO_3: 268, 372 nm, + NaOMe: 282, 438 nm, + $AlCl_3$: 268, 302 (sh), 426 nm, + $AlCl_3$/HCl: 268, 302 (sh), 428 nm. Its identity was confirmed by comparing the UV data with that of authentic kaempferol[32,33], Co-chromatography and m.m.p.

Kaempferol-3-methyl ether: Yellow residue (Ether), UV λ_{max}^{MeOH}: 268, 298, 350 nm, + NaOAC: 278, 374 nm, + NaOAC/H_3BO_3: 268, 354 nm, + NaOMe: 270, 302 (sh), 402 nm; + $AlCl_3$: 268, 305 (sh), 398 nm, + $AlCl_3$/HCl: 269, 305 (sh), 396 nm. Its identity was confirmed by comparing the UV data [32,33], Co-chromatography and m.m.p. with authentic sample.

Quercetin: Yellow residue (MeOH) m.p. 315°C, UV λ_{max}^{MeOH}, 260, 270 (sh), 376 nm, + NaOAC: 276, 324, 388 nm, + NaOAC/H_3BO_3: 264, 392, 440(sh), nm, + NaOMe: 280(sh), 330, 400 nm, + $AlCl_3$: 270, 300 (sh), 442 nm; $AlCl_3$/HCl: 266, 300 (sh), 426 nm. Identification was confirmed by comparison with published data[32,33], Co-chromatography and m.m.p. with authentic sample.

Apigenin: Pale-yellow residue (Acetone), m.p. 348-50°C, UV λ_{max}^{MeOH}: 268, 336 nm, + NaOAC: 276, 300 (sh), 370 nm, + NaOAC/H_3BO_3: 268, 340 nm, + NaOMe: 274, 326, 394 nm, + $AlCl_3$: 276, 304, 348, 384 nm, + $AlCl_3$/HCl: 276, 300 (sh), 344 nm. Identification was confirmed by comparison with published data[32,33], Co-chromatography and m.m.p. with authentic sample.

Isoquercitrin: Yellow amorphous substance (EtOH), m.p. 183-85°C, UV λ_{max}^{MeOH}: 260, 356 nm, + NaOAC: 268, 324 (sh), 384 nm, + NaOAC/H_3BO_3: 264, 376 nm, + NaOMe: 270, 328 (sh), 408 nm, + $AlCl_3$: 270, 300 (sh), 414 nm, + $AlCl_3$/HCl: 270, 300 (sh), 356, 400 nm. Acid hydrolysis yielded quercetin (identified by UV and co-chromatography), and glucose.

Alkaloids and Nitrogenous Bases:

Siaminine B.: Yellow residue, HCl salt m.p. 235°C, UV λ_{max}^{MeOH}, 230, 245, 252, 297, 337, 355 nm. IR(KBr): 3350, 3100, 2800, 1630, 1600, 1410, 1390, 1280, 1160, 1115, 910 & 835 cm^{-1}. Its identity was confirmed by comparing the UV data, IR spectrum, m.m.p. and Co-chromatography with the alkaloid previously isolated from the leaves of, C. siamea Lam.[26]

Didymobotrine: Yellow plates ($CHCl_3$), m.p. 270-72°C; UV λ_{max}^{MeOH}: 290, 354 nm, IR (KBr): 3550, 3090, 2960, 1625, 1615, 1560, 1555, 1500, 1480, 940 & 960 cm^{-1}. Identified by comparing the obtained results with that for the alkaloid previously is-

olated from the leaves of C.didymobotrya Fres.[25].

Choline: Prepared as rheineckate, m.p. 274°C, insoluble in water, chloroform, soluble in acetone and methanol, decomposition with silver nitrate gave yellow ppt. Identification was verified by undepressed m.m.p. and superimposable IR spectra with authentic choline base.

Chromone:

Cassiachromone: Colourless needles (Acetone), m.p. 208-209°C, UV λ_{max}^{MeOH} : 212, 243, 251, 300 nm, + NaOAC: 255, 300 (sh), 330 nm, IR (KBr): 3200, 1720, 1640, 1622, 1575, 1150 & 730 cm^{-1}. Identified by comparying UV & IR spectra, m.m.p., and Co-chromatography with that previously isolated from C. siamea Lam. leaves[23].

Leuco-anthocyanin: Amorphous, pale brown (Acetone), hydrolysis with hydrochloric acid revealed the presence of pelargonidin HCl,[34,35] (PC red spot, UV λ_{max}^{MeOH} : 520 nm, + $AlCl_3$: no shift).

Table 1: Physical and chemical characteristics of the studied seed oils of different cassia species .

Characters	*C.javanica*	*C.siamea*	*C.fistula*	*C. didymobotrya*
Colour	dark-yellow	yellow	y. brown	y. brown
Odour	slight	slight	slight	slight
Taste	sl. bitter	sl. bitter	sl. bitter	sl. bitter
Refractive index	1.455	1.483	1.445	1.470
Acid value	27.620	33.660	15.976	28.050
Iodine value	98.040	89.920	88.830	85.230
Saponification value	191.700	194.300	182.060	185.300
% of the oil	10.560	11.830	10.500	12.300
Unsaponifiable matter (%)	2.810	2.600	1.920	2.270
Unsaturated fatty acids (%)	58.76	70.700	33.930	53.700
Saturated fatty acids (%)	39.84	27.38	64.51	44.000

Table 2: G.L.C. Analysis of the fatty acid methyl esters of the studied seed oils of different Cassia species.

Peak No.	Carbon No. & No. of π bonds	Fatty acid methyl ester	Weight % in C.javanica	C.siamea	C.fistula	C.didymobotrya
1	12:0	Lauric	3.73	—	6.49	-
2	14:0	Myristic	8.81	7.3	13.02	15.2
3	14:1	Myristoleic	0.96	tra.	11.05	tra.
4	16:0	Palmitic	19.70	20.08	32.30	22.0
5	16:1	Palmitoleic	tra.	4.80	—	tra.
6	16:2	Palmitolenic	1.80	—	—	0.9
7	18.0	Stearic	7.60	tra.	12.70	6.8
8	18.1	Oleic	10.50	0.90	9.80	30.2
9	18:2	Linoleic	30.20	43.70	—	22.6
10	18:3	Linolenic	15.30	21.30	—	—
11	20:4	Arachidonic	traces	—	13.08	traces

Table 3: Different components isolated from the fruit pericarps of the studied Cassia species.

Component	*C.javanica*	*C.siamea*	*C.fistula*	*C.didymobotra*
Heptacosane	+	+	–	+
Hentriacontane	+	+	–	–
Cerotic acid	+	+	–	–
Unidentified long chain alcohol	–	–	+	–
B-sitosterol	+	+	+	+
∝-amyrin	+	+	+	+
B-amyrin	+	+	–	–
Unidentified sterol	+	–	–	–
Chrysophanol	–	+	+	+
Aloe-emodin	–	–	+	+
Emodin	+	–	–	–
Physcion	+	+	+	–
Rhein	Traces	–	+	+
Rhein-glycosides	–	–	+	+
Unidentified anthraquinone	+	–	+	–
Kaempferol	+	–	+	+
Kaempferol 3-methyl ether	+	–	–	–
Quercetin	–	–	traces	+
Apigenin	–	traces	–	–
Isoquercitrin	–	–	–	+
Unifentified flavonoid	+	traces	traces	+
Alkaloids	–	+	–	+
Nitrogenous bases	–	–	–	+
Leuco anthocyanin	+	–	–	–
Chromones	–	+	–	–

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دراسة كيميائية لثمار بعض انواع نباتات الكاسيا المنزرعة فى مصر

سامية محمد الصياد ـ هناء محمد سيد
كلية الصيدلة ـ قسم العقاقير ـ جامعة اسيوط

لثمار معظم نباتات الكاسيا عدة استعمالات طبية معروفة لذا وجد انه من الضرورى دراسة محتوياتها من المود الفعالة.

وقد تم فى هذا البحث دراسة كيميائية لثمار اشجار الكاسيا جافانيكا (لن) والكاسيا سياميا (لام) والكاسيا فستيولا (ل) (الخيا× شمر) وشجيرة الكاسيا ديديموبتريا (فرس).

وقد اثبت المسح الكيميائى لها وجود مواد فلافونيدية وانثراكينونية وكرومونات وقلويدات واستيرولات غير مشبعة وكذلك تربينات ثلاثية وقد تم فصل هذه المواد من الثمار المختلفة وبدراستها كيميائيا وفيزيائيا وكروماتوجرافيا ومقارنتها بالمواد المفصولة من قبل أمكن التعرف عليها وقد اثبتت هذه الدراسة وجود قلونيات فى كل من الكاسيا سياميا (لام) والكاسيا ديدييموبتريا (فرس) فقط.

كذلك تم دراسة الزيت فى كل من البذور المفصولة من الثمار المختلفة وحددت صفاته وثوابته. وقد أمكن التعرف على الاحماض الدهنية عن طريق كروماتوجرافيا الغاز المشتق المثيلى لاسترات هذه الاحماض.

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