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PHYTOCHEMICAL STUDY OF *CLERODENDRON INERME* L. GROWING IN EGYPT

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ABSTRACT

Phytochemical study of the leaves and stems of *Clerodendron inerme* L. resulted in the isolation of five flavonoidal compounds; apigenin, apigenin 7-O-glucoside, luteolin, luteolin-7-O-glucoside, kaempferol and one coumarin compound; scopoletin. The structures of isolated compounds have been established on the bases of, chromatographic and spectral studies. In addition three triterpenoidal saponin compounds have been also isolated ß-amyrin and oleanolic acid were found to be the aglycones of the isolated saponins. The percentage of the total saponins were found to be 2.62% in the leaves and 0.84% in the stems as determined by haemolytic index method.

INTRODUCTION

Clerodendron inerme L.Family Verbenaceae[1,2] is a tall shrub or small tree with white flowers and opposite, obovate leaves. It is distributed throughout India[3] and warm regions[4] of the world. The plant has been acclimatised and grown in Egypt for the ornamental purposes. In folk medicine the plant was used in the treatment of rheumatism and to resolve the bubos[5]. The alcoholic extract of the plant was reported to have uterotonic, hypertensive and laxative effects[6]. Recently, the flavonoidal compounds were reported to have antiinflammatory activity[7]. Previous work on *C. inerme* leaves reported the isolation of flavonoids[7,8]; sorbifoline; pectolinarigenin, apigenin, acacetin and salvigenin, monoterpene alkaloids, bitter principles[9]; and diterpenes[10]. Nothing was reported concerning the saponin and coumarin contents of the plant.

Since, soil and climatic variations play an important role in determining the nature of active constituents, the present study had been undertaken to investigate and characterise coumarins, flavonoids and saonins contents of *C. inerme* L. growing in Egypt to complete the phytochemical picture of the plant.

EXPERIMENTAL

General Experimental Procedures:

Melting points were determined using heating stage Kolfer's microscope, U.V. spectra were determined in methanol on a Unicam Sp. 8000 spectrophotometer. MS was carried out on Varian/MAT 1125 mass spectrometer attached to Varian data machine Model 76, U.S.A. IR spectra were obtained on a Beckman spectrophotometer. NMR spectrum was recorded on Varian-XLL-300. Chromatography was carried out on silica gel GF 254 (Merck) for TLC and on Whatmann No. 1 and 3 mm filter paper for PC[11,12].

Several solvent systems were tried, the best solvents were acetic acid-water 15:85 and n-butanol-acetic acid-water (6:1:2) (PC, for flavonoids and coumarins); n-butanol-acetic acid-water (4:1.5:0.5) (TLC, for flavonoids); n-butanol-acetic acid-water (4:1:5) (PC, for sugars) and Chloroform-methanol-water (65:35:10) (TLC, for saponins). The spots were detected by, spray reagents: 1% aluminium chloride in methanol for flavonoid, aniline phthalate for sugars and Modified Khagi Mischner reagent for saponins. Reference materials for flavonoids: coumarins, genins of saponins and sugars were obtained from their respective sources, only white saponin (Merck) was used as authentic sample for saponins.

Plant Material:

The aerial parts of *Clerodendron inerme* L. were collected in May 1991 from the Experimental Station of Medicinal Plant, Faculty of Pharmacy, Cairo University. Fresh leaves and stems were separately air dried in shade and reduced to powder form. The identity of the plant material was kindly authenticated by Dr. Nabil El-Hadidi, Professor of Plant Taxonomy, Faculty of Science, Cairo University.

Extraction and Isolation Procedures of the Flavonoids, Coumarins and Saponins:

Air dried powder of leaves and stems (500 g each) were seperately exhaustively extracted by successive portions of alcohol 95% followed by alcohol 70%. Both extracts were combined and evaporated under vacuum (45°C). The residue was dissolved in boiling water and filtered while hot. The filtrate was successively extracted with petroleum ether, ether and ethyl acetate. The different extracts were concentrated under vacuum, dried over anhydrous sodium sulphate and kept for investigation of their flavonoids and coumarins content. The remaining aqueous extract was lyophilized, dissolved in methanol and kept for investigation of their flavonoid and saponin contents.

1- Investigation of Flavonoids and Coumarin:

The prepared extracts of *C. inerme* were spotted alongside with reference samples on both PC and TLC and developed using the previously mentioned solvent systems for PC and TLC. Visualisation was achieved by UV λ 365 nm, NH_3-UV λ 365 nm and $AlCl_3$-UV λ 365 nm. The developed chromatograms showed qualitative similarity between leaves and stems extracts with three spots in the ether extracts and another three spots in the ethyl acetate extracts. Isolation was achieved from leaves.

The concentrated ether extract was segregated through preparative paper chromatography whatmann III MM and developed. The eluted bands (R_f 0.53, 0.12 and 0.06) were separately concentrated, monitored by PC and crystallized from methanol to yield compounds A,B and C, respectively. The concentrated ethyl acetate extract were treated as the ethereal extract. The eluted bands (R_f 0.76, 0.60 and 0.40) were separately concentrated, monitored by PC and crystallized from methanol to yield compounds D, E and F respectively. The identity of the isolated compounds was achieved by m.p., m.m.p., co-chromatography with authentic samples and comparing their spectral data with the previously cited literature for authentics[13].

Compounds E and F, 5 mg each were separately refluxed with N/2 H_2SO_4 (2 ml) for 2 hours. The aglycone of each was separately extracted with ether, concentrated, monitored by TLC and identified by IR, m.p., m.m.p. and UV spectroscopic methods. The aqueous layer was neutralized by barium carbonate, filtered, lyophilized and the sugar was dissolved in 10% isopropyl alcohol and monitored by PC alongside with authentic sugars using the solvent system n-butanol-acetic acid-water (4:1:5) and aniline phthalate as spraying reagent.

Compound (A):

White needles [15 mg] with Rf 0.53 (n-butanol-acetic acid-water 6:1:2, m.p. 203-204°C. UV methanol, λ_{max} (nm) 225, 254, 260, 260, 294 and 342. ^{1}H-NMR (δ, $CDCl_3$, 300 MHz) 7.6 (1H, d, J = 9Hz) 6.9 (1H,s), 6.8 (1H,s), 6.2 (1H, d, J = 9Hz), 3.9 (3H,s) Ms m/z 192 (M^+, 100%) calculated for $C_{10}H_8O_4$, 177 (52%), 164 (M-CO; 26.38%), 149 (45.7%), 121 (20.35%) and 69 (23.91%).

Compound (B):

Yellow needles [30 mg] with R_f 0.20 acetic acid-water (15:85); m.p. 347-349°C; UV methanol and shift reagents λ_{max} (nm) MeOH 265, 334; $NaOCH_3$ 272, 320, 392; $AlCl_3$ 276, 301, 348, 384; $AlCl_3$/HCl 276, 299, 384; NaOAC 274, 301, 375; NaOAC/H_3BO_3 268, 302 sh, 338.

Compound (C):

Yellow needles [20 mg] with R_f 0.06 acetic acid-water (15:85); m.p. 330°C; UV methanol and shift reagents λ_{max} nm MeOH 253, 267, 294 sh, 349; $NaOCH_3$ 266 sh, 325 sh, 401; AlCl3 275, 300 sh, 328, 425; $AlCl_3$/HCl 266 sh, 275, 294 sh, 355, 385; NaOAC 269, 326 sh, 348, NaOAC/H_3BO_3 259, 301 sh, 370, 430 sh.

Compound (D):

Yellow needles [15 mg] with R_f 0.75 (n-butanol-acetic acid-water (6:1:2); m.p. 278-280°C; UV methanol and shift reagents λ_{max} (nm) MeOH 267, 294 sh, 366; $NaOCH_3$ 277, 313, 415; $AlCl_3$ 268, 303 sh; 350, 424; $AlCl_3$/HCl 267, 305 sh, 348, 424; NaOAC 274, 303, 387; NaOAC/H_3BO_3 267, 372.

Compound (E):

Yellow needles [35 mg], R_f 0.6, n-butanol-acetic acid-water (6:1:2), m.p. 177-180°C. UV using methanol and shift reagents λ_{max} (nm) MeOH 268, 330, $NaOCH_3$ 245 sh, 267, 301 sh, 386 $AlCl_3$ 276, 300, 348, 386 $AlCl_3$/HCl 277, 229, 344, 382 NaOAC 267, 355, 387 NoAC/H_3BO_3 267, 340.

Compound (F):

Yellow needles [5 mg], Rf 0.40, n-butanol-acetic acid-water (6:1:2), m.p. 254-256°C. UV using methanol and shift reagent λ_{max} nm MeOH 255, 267 sh, 345 $NaOACH_3$ 263, 300 sh, 394 $AlCl_3$ 274, 298 sh, 329, 432 $AlCl_3$/Hcl 273, 294 sh, 358, 387 NaOAC 259, 266 sh, 365 sh, 405 NaOAC/H_3BO_3 259, 372.

2- Investigation of Saponin Content:

The methanol solutions of the lyophilized residue of the aqueous extracts remaining after extraction of flavonoids and coumarins were separately poured over a large volume of ether to precipitate the crude saponin. The process is repeated to obtain pure crude saponin. TLC examination of the saponins of both leaves and stems using silica gel GF 254 and chloroform-methanol-water (65:35:10) as a developing solvent system and modified Khagi reagent as visualizing reagent showed that the crude saponins of leaves and stems have the same chromatographic pattern. The crude saponin mixture (3 g) of leaves was applied on the top of VLC column (20 g, 2 x 5 cm), the elution started with chloroform and the polarity increased by addition of methanol. Fractions, 100 ml,

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each were collected, concentrated and tested for saponin by froth and haemolysis tests and monitored by TLC as previously mentioned. The fractions 41-80 ($CHCl_3$: MeOH, 95:5) showed two spots on TLC (R_f 0.88 and 0.85). They gave yellowish brown and violet colour, respectively with modified Khagi reagent. Only one pure saponin compound (R_f 0.85) could be isolated adopting PTLC using solvent system chloroform-methanol-water (65:35:10). The corresponding band eluted with 70% ethanol to give compound G (300 mg).

The Fractions 81-140 ($CHCl_3$: MeOH, 90:10) gave two main spots (R_f 0.85 and 0.77, respectively), gave violet colour with Khagi reagent. Two saponin compounds could be separated adopting PTLC as previously done to give compound G, R_f (0.85 mg) and compound H, R_f 0.77 (250 mg).

The Fractions 145-200 ($CHCl_3$: MeOH, 86:14) showed two main sots on TLC (R_f 0.66 and 0.6). They gave blue and olive green colour respectively with Khagi reagent. The pooled fractions were subjected to PTLC to give saponin compound I, R_f 0.6 (280 mg).

Study of the Hydrolysates:

About 0.2 g of compounds G, H and I were separately refluxed with 7% sulphuric acid in 50% aqueous ethanol for 7 hours. The aglycone of each saponin glycoside was separately extracted with $CHCl_3$, leaving the free sugars in the aqueous layer. The obtained pure aglycones were identified by comparing their m.p., chromatographic picture and I.R. spectral, data with that obtained for authentic samples.

The acidic aqueous layer of each hydrolysate was separately neutralised with $Ba(CO_3)_2$, filtered, then lyophilized, the suagrs were dissolved in isopropyl alcohol, analysed on PC alongside with authentic suagrs using solvent system n-butanol-acetic acid-water (4:1:5) and aniline phthalate as spray reagent.

Compound (G):

This compound appeared as a violet spot on TLC [R_f 0.85, chloroform-methanol-water (65:35:10)] with modified Khagi reagent. Acid hydrolysis of the compound G afforded white needles (20 mg), m.p. 306°C, infrared was carried out in KBr. Paper chromatography of acidic aqueous hydrolysate solution gave one brown spot, [R_f 0.2, n-butanol-acetic acid-water (4:1:5)] on spraying with aniline phthalate.

Compound (H):

This compound appeared as a violet spot on TLC, R_f 0.77, using solvent system chloroform-methanol-water (65:35:10) with modified Khagi reagent. Acid hydrolysis of compound H gave white needles (12 mg), m.p. 306°C, infra red was run in KBr. Paper chromatography of the acidic aqueous hydrolysate solution gave two brown spots (R_f 0.44 and 0.22, n-butanol-acetic acid-water (4:1:5)] on spraying with aniline phthalate.

Compound (I):

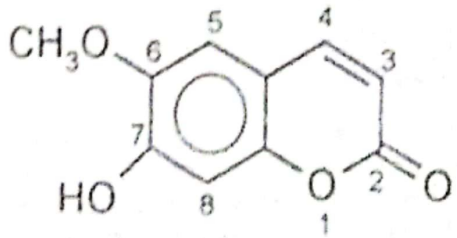
This compound appeared as olive green spot on TLC [R_f 0.6, chloroform-methanol-water (65:35:10)] with modified Khagi reagent. Acid hydrolysis of compound I afforded a crystalline substance (20 mg) which appeared as reddish-violet spot, m.p. 196-198°C, infrared was carried out in KBr. Paper chromatography of the acidic aqueous hydrolysate solution gave two brown spots [R_f 0.04, 0.44; n-butanol-acetic acid water (4:1:40)], with aniline phthlate reagent.

Estimation of Saponin Content:

The saponin content of both leaf and stem was estimated adopting the haemolytic index method[10,11].

RESULTS AND DISCUSSION

The Phytochemical study of the ether and ethyl acetate extracts of the defatted powdered leaves of *C. inerme*, exhibited the presence of one coumarin compound and five flavonoids. The identification of these compounds was based on comparison of their m.p., chromatographic behaviour and spectral data with the data reported for reference compounds.



Compound A:

The blue colour of the compound in the UV (intensified with NH_3) indicated the coumarin nature of compound A. The ^{1}H-NMR showed two doublets with $J = 9$ Hz centered at δ 6.2 and 7.6 assigned for C_4 and C_3 protons strongly indicates a coumarin unsubstituted in the pyrone ring. The occurrence of the two aromatic singlets at δ 6.8 and 6.9 indicates that the aromatic ring is substituted at C_6 and C_7. The comparison of spectral data (UV, ^{1}H-nmr and Ms) and the chromatographic behaviour of compound A with those of authentic scopuletin proved that the two compounds were identitical. This suggested that the compound A is scopletin.

The identity of compound B was confirmed by comparing its UV spectral data, mp, mmp and co-chromatography with that published for apigenin.

Compound C:

Data are identical with those for luteolin, indicating that compound C is luteolin.

Compound D:

Compound D was found to be identical with Kaempferol (mp, mmp, co-chromatography) and comparing the UV spectral data with that of the published data for kaempferol[13]. This indicates that compound D is Kaempferol.

Compound E:

The compound appeared as a dark purple spot on paper chromatography under UV and converted to yellow colour with ammonia and aluminium chloride. The positive reaction of the compound E before hydrolysis with fehling's solution indicated that compound E is a glycoside. The UV showed a band I absorption at 330 nm (MeOH) indicating a flavone structure. The site of glycosilation ws found to be 7-OH as indicated by a bathochromic shift in band II (9 nm) with fused Na acetate after hydrolysis (no effect before hydrolysis hydrolysis of compound E gave an aglycone identifical to apigenin (mp, mmp co-chromatography) and comparing the UV spectral data with that of the published data of authentic sample[13]. The sugar moiety was found to be glucose as shown by co-chromatography with reference glucose, so compound E was suggested to be apigenin-7-O-glucoside.

Compound F:

This compound appeared as a dark purple spot on paper chromatography under UV and converted to yellow colour with ammonia and aluminium chloride. The UV spectral data using methanol suggested (band I, 348) the flavone structure of compound F. The 3',4'-dihydroxyl groups were proved from the hypsochromic shift in band I (70 nm) $AlCl_3$/HCl compared to band I in $AlCl_3$, and bathochromic shift in band I in fused Na acetate/Boric acid. Free C-5OH (bathochromic shift in band II in $AlCl_3$. The site of glycosidation was found to be at 7-OH as indicated by the bathochromic shift in band II with fused Na acetate after hydrolysis in comparison with no effect before hydrolysis of compound F. Compound F gave an aglycone identical to Luteolin (m.p., m.m.p., co-chromatography and comparing is UV spectral data with that of published data)[13]. The sugar moiety was shown to be glucose as proved by co-chromatography with reference glucose. So compound F was suggested to be luteolin-7-O-glucoside.

Concerning saponins, three saponin glycosides G, H and I were isolated adopting vacuum liquid chromatography, followed by PTLC [(R_f 0.85, 0.77 and 0.6 respectively, chloroform-methanol-water (65:35:10)]. The compounds G and H on acid hydrolysis produced the same aglycone as tested by TLC [(Rf 0.51, benzene-ethyl acetate (86:14)]. This aglycone was found to be identical to oleanolic acid (m.p., mixed m.p., IR, co-chromatography). The acidic aqueous solution of the hydrolysate of compound G and H were separately investigated by P.C. for their sugar moities alongside with authentic sugars. The sugar moiety of compound G was found to be identifcal to galactose while that of compound H was found to be identifcal to rhamnose and galactose. This indicated that the sugar moiety of compound G is galactose while that of compound H is rhamnose and galactose. Compound I, gave positive test for saponin and positive Molisch's test. It revealed one spot on TLC [R_f 0.6, chroroform-methanol-water (65:35:10)]. The acid hydrolysis of compound I produced an aglycone. It showed one olive green spot on TLC examinations [(R_f 0.58, benzene-ethyl acetate (86:14)] with modified Khagi reagent. It was found to be identical with B-amyrin (m.p., mixed m.p., IR and co-chromatography). This indicating that the aglycone of compound I is ß-amyrin. The suagr moiety of compound I was investigated by P.C. (R_f 0.04 and 0.44) alongside with authentic sugars. It was found to be identical with galacturonic acid and rhamnose respectively. This indicating that the sugar moiety of compound I are galacturonic acid and rhamnose. Estimation of saponin content in *C. inerme* applying haemolytic index method showed that the leaves have higher saponin contents (2.62%) than the stem saponin content (0.84%).

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دراسة فيتوكيميائية لنبات الياسمين الزفر الذى ينمو فى مصر

على محمد الشامى - عبد الرحمن الشبراوى - نبوية مصطفى الفقى
قسم العقاقير - كلية الصيدلة - جامعة القاهرة

أثبتت الدراسة الكيميائية لنبات الياسمين الزفر الذى ينمو فى مصر عن فصل خمس مركبات فلافونيدية وهى ابيجينين، ابيجنين ٧-أ جلوكوزيد، ليتولين، وليتولين ٧-أ جليكوزيد والكامفيرول. تم فصل مادة كومارينية وهى اسكوبوليتين وقد تم التعرف على هذه المواد بمقارنتها بمواد أصيلة.

علاوة على ذلك فقد تم دراسة المحتوى الصابونينى لهذا النبات . وتم فصل ثلاثة مركبات صابونينية. وقد تم التعرف على هذه المواد بأنها جلكوزيدات للبيتا اميرين وحمض الأوليانوليك وكذلك تم تحديد نسبتها فى النبات وأثبتت الدراسة أن نسبة الصابونين فى الأوراق ٢ر٦٢٪ وفى السيقان ٨٤ر٠٪ بطريقة دراسة معامل تحلل كرات الدم الحمراء.