

FLAVONOIDAL CONTENTS OF *ALESTONIA SCHOLARIS* R.Br.

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نبات الأستونيا شولاريز التابع للعائلة الدفلية هو من الأشجار كبيرة الحجم دائمة الخضرة وينمو عادة في الهند ودول جنوب شرق آسيا ويعرف باسم "قشور ديتا" أو "قشور ديفل". ولهذا النبات عدة استعمالات في الطب الشعبي حيث أن أوراقه وقشوره تستخدم كمواد قابضة وكمقويات وكمواد طاردة للديدان ويستخدم أيضا في علاج حالات الحمى وفي علاج بعض الأمراض المعدية. وللنبات أيضا استخدامات متعددة في علاج بعض أنواع الروماتزم. وتزرع هذه الشجرة في مصر في جزيرة النباتات بأسوان ومنها حصلنا على الأوراق لأجراء هذه الدراسة.

وباستقصاء المراجع المتواترة تبين أن الغالبية العظمى من الأبحاث المنشورة والتي أجريت على هذا النبات تختص بالمحتوى القلوي وليس هناك أية دراسات تتصل بالمكونات الفعالة الأخرى الموجودة في النبات، وهذا ما حدا بالباحث لإجراء دراسة للمحتوى الفلافونيدى لهذا النبات حيث تم فصل والتعرف على أربعة جليكوزيدات فلافونيدية يتم فصلها لأول مرة من هذا النبات وهي: الأيزورامنتين-3-أ-جالاكتوبيرانوزيد، الكامفيرول-3-أ-جلوكوبيرانوزيد، الأيزورامنتين-3-أ-جلوكوبيرانوزيد والكامفيرول-3-أ-2-رامنوزيل-روتينوزيد) بالإضافة إلى الأيزورامنتين والكامفيرول.

Four flavonoidal glycosides; viz. isorhamnetin-3-O- β -D-galactopyranoside (A), kaempferol-3-O- β -D-glucopyranoside (B), isorhamnetin-3-O- β -D-glucopyranoside (C) and kaempferol-3-O-(2⁸)-rhamnosylrutinoside (D), in addition to the flavonoidal aglycones: Isorhamnetin and kaempferol have been isolated from the leaves of *Alestonia scholaris* R.Br. (Fam. Apocynaceae) for the first time.

The structures of the isolated compounds were principally deduced by physical and chromatographic characters as well as by spectroscopic analyses.

INTRODUCTION

Alestonia scholaris R.Br. (Apocynaceae)¹ is a tall evergreen tree growing widely in India, in sub-Himalayan areas and through S.E. Asia to Indochina and known as Dita bark or Devil's bark. The plant is cultivated in the Botanic Island in Aswan (Upper Egypt). It has been widely used in folk medicine as an astringent, tonic, anthelmintic, antiperiodic in fevers, in restoring stomach muscle after debility due to fevers and in treatment of some forms of rheumatism.¹

Reviewing the available literature, nearly all publications on *Alestonia scholaris* R.Br. and other species of *Alestonia* are concerned with the alkaloidal contents (indole type) of the plant,²⁻⁴

but nothing could be traced about the other contents.

The present study includes the isolation and identification of four flavonoidal glycosides and two flavonoidal aglycones from the plant.

EXPERIMENTAL

Plant material

The leaves of *Alestonia scholaris* R.Br. were collected in April 1997 from the trees growing in the Botanic Island in Aswan (Upper Egypt). The plant was identified and authenticated by Prof. A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University to whom we are indebted. A voucher sample is kept in Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.

General experimental procedures

Melting points were uncorrected. ¹HNMR spectra were recorded in DMSO using varian spectrometer (60 MHz) using TMS as internal standard. Thin layer chromatography was carried out on silica gel G plates (kieselgel 60 F₂₅₄, E. Merck) and for column chromatography, silica gel (E. Merck, 230-400 mesh) was used. Preparative paper chromatography was carried out on Whatmann paper No. 3. Spray reagents: AlCl₃ (for flavonoids) and triphenyltetrazolium chloride (for sugars) were used. Authentic samples of the isolated substances (aglycones, glycosides and sugars) were obtained from Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.

Extraction and isolation

The air-dried leaves (1 kg) of *Alestonia scholaris* R.Br. were extracted with ethanol (70%) till exhaustion. The alcohol extract was concentrated under reduced pressure till nearly dry. The syrupy extract (230 g) was defatted with n-hexane and successively exhausted with chloroform, ethyl acetate and n-butanol.

The chloroformic extract (11 g) was chromatographed over silica gel column (350 g, 5 x 150 cm) using chloroform-methanol mixture in a manner of increasing polarities. Fractions (200 ml, each) were collected and monitored informing the flavonoidal compounds by TLC and inspection of the developed plates was under UV light at (366, 254 nm) as well as by spraying with AlCl₃ and 10% H₂SO₄. Similar fractions were collected and the fractions eluted with chloroform-methanol (95:5) afforded isorhamnetin and kaempferol (both were identified by comparison with authentic samples due to their minor quantities).

The ethyl acetate extract (9.5 g) was chromatographed over a column of silica gel (300 g, 5x150 cm) and eluted with ethyl acetate-methanol in order of increasing polarities followed by rechromatography of the obtained fractions with smaller sub-columns and preparative paper chromatography using 15% HOAc and BAW (4:1:2) afforded the isolation of the compounds (A-C).

The n-butanol extract (20 g) was chromatographed over silica gel column (500 g, 5 x 200 cm) afforded the isolation of compound (D) in pure form.

Compound (A): Isorhamnetin-3-O-β-D-galactopyranoside, yellow powder from MeOH (89 mg), R_f: 0.25 (ethyl acetate - methanol - water, 85:15:1). ¹HNMR: see Table 1.

Compound (B): Kaempferol-3-O-β-D-glucopyranoside, yellow powder from MeOH (136 mg), R_f: 0.24 (chloroform - methanol - water, 85:15:1). ¹HNMR: see Table 1.

Compound (C): Isorhamnetin-3-O-β-D-glucopyranoside, yellow needles from MeOH (110 mg), m.p. 115°C, R_f: 0.24 (ethyl acetate - methanol-water, 85:15:1). ¹HNMR: see Table 1.

Compound (D): Kaempferol-3-O-(2^β-rhamnosylrutinoside), yellow needles from MeOH (65 mg), m.p. 190°C, R_f: 0.14 (chloroform - methanol - water, 65:35:5). ¹HNMR: see Table 1.

Acid hydrolysis of the isolated glycosides

Each isolated glycoside (20 mg) was dissolved in N/2 H₂SO₄, mixed with an equal volume of ethanol and refluxed for 2 hours. The reaction mixture was diluted with water and extracted with chloroform. The aglycone was isolated from the chloroform extract and subjected for TLC alongside with reference aglycones. The aqueous layer was neutralized with barium carbonate, filtered and subjected to TLC on silica gel using chloroform-methanol-water (6:4:1) as solvent system and detection with triphenyltetrazolium chloride reagent.¹⁰ R_f values were: 0.15, 0.17 and 0.32 for D-galactose, D-glucose and L-rhamnose respectively.

RESULTS AND DISCUSSION

The alcoholic extract of the air-dried powdered leaves of *Alestonia scholaris* was defatted with n-hexane and successively exhausted with chloroform, ethyl acetate and n-butanol. The chloroformic extract was chromatographed over silica gel column to produce two flavonoidal aglycones with minor

Table 1: ¹HNMR spectral analysis of the isolated glycosides, DMSO, TMS as internal standard.

Proton	A	B	C	D
H-6	6.20, d, J= 2.2	6.20, d, J= 2.02	6.21, d, J= 2.01	6.18, d, J= 2.02
H-8	6.50, d, J= 2.2	6.43, d, J= 2.01	6.45, d, J= 2.02	6.38, d, J= 2.02
H-2'	8.00, d, J= 2.02	8.03, d, J= 8.8	7.94, d, J= 2.01	7.94, d, J= 6.96
H-3'	--	6.88, d, J= 9.00	--	6.86, d, J= 6.77
H-5'	6.90, d, J= 8.43	6.88, d, J= 9.00	6.92, d, J= 8.42	6.86, d, J= 6.77
H-6'	7.50, dd, J= 2.01, 8.40	8.08, d, J= 8.8	7.50, dd, J= 2.02, 8.43	--
OCH ₃	3.85s	--	3.85 s	--
H-1-glc	--	5.45, d, J= 7.33	5.57, d, J= 7.5	5.48, d, J= 7.33
H-1-rha	--	--	--	5.05, bs, & 4.30 bs
H-1-gal	5.50, d, J= 7.7	--	--	--
CH ₃ -rha	--	--	--	0.95, d, J= 6.23 & 0.80, d, J= 6.23

glc. = glucose, rha. = rhamnose, gal. = galactose.

quantities which have been identified as isorhamnetin and kaempferol on the basis of physical, chemical and chromatographic characters when compared with authentic samples.^{5,6}

Column chromatography of the ethyl acetate extract over silica gel followed by preparative paper chromatography afforded three compounds (A-C). Also, by chromatographing the n-butanol extract on silica gel column and preparative paper chromatography, the compound (D) has been isolated. The physical, chemical and chromatographic characters of the isolated compounds (A-D) proved their nature as flavonoid glycosides.^{5,8}

¹HNMR of compound (A) (Table 1) showed two doublets at δ 6.50 and 6.20 ($J = 2.2$ Hz), characteristic for H-8 and H-6 respectively as well as other two doublets at δ 8.00 and 6.90 for H-2' and H-5' respectively, in addition to a signal at δ 7.50 (dd) for H-6' indicating the substitution of ring-B at C-3' and C-4'. The signal at δ 3.85 indicated the presence of a methoxy group at C-3'.

The inspection of these signals of compound (A) revealed the same chemical shifts of 3-O-substituted isorhamnetin.⁷

The signal appearing at δ 5.50 (d, $J = 5.7$ Hz) is related to the anomeric proton of galactose.^{8,9}

The acid hydrolysis of compound (A) yielded isorhamnetin in addition to D-galactose which was identified by TLC alongside with an authentic sample.

Comparison of ¹HNMR spectrum of compound (C) with that of compound (A) revealed their similarity except the chemical shift of the sugar part which appeared at δ 5.57 (d, $J = 7.5$ Hz).

From the above-mentioned data, in addition to comparison with authentic samples, compound (A) was assigned as isorhamnetin-3-O- β -D-galactopyranoside while compound (C) was assigned as isorhamnetin-3-O- β -D-glucopyranoside.⁷

¹HNMR spectra of compounds (B), (D) were found to be similar except the signals of the sugar moieties. The two doublets at δ

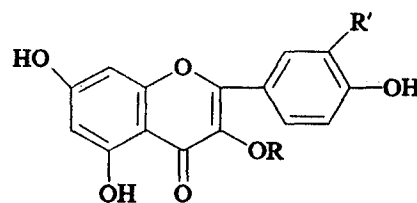
$\sim 8.00, 6.87$ for H-2', H-6' and H-3', H-5' respectively, forming AA', BB' system characteristic for a P-substituted ring, provided a further support for absence of substitution at C-3'. On the other hand, the acid hydrolysis of the forementioned compounds gave kaempferol in addition to the other sugar moieties.^{7,10}

Compound (B) showed an anomeric proton signals in its ¹HNMR spectrum at δ 5.45 revealing the presence of only one β -D-glucose moiety in the molecule. It was deduced to be kaempferol-3-O- β -D-glucopyranoside.

Compound (D) showed two anomeric proton signals at δ 5.05 and 4.31 which are attributed to two rhamnose moieties and another β -D-glucose anomeric proton signals appearing at δ 5.48 revealing that compound (D) is kaempferol-3-O-(2^β-rhamnosylrutinoside).¹⁰

Reviewing the current literature, it was found that the isolated glycosides (A-D) are reported for the first time in *Alestonia scholaris* R.Br.

The structural formulae, from the obtained data, for the compounds (A-D) are illustrated in Figure (1).



Compound	R	R'
A	β -D-gal.	OCH ₃
B	β -D-glc	H
C	β -D-glc	OCH ₃
D	β -D-glc α -L-rha α -L-rha	H

Fig. 1: The structural formulae of the isolated compounds (A-D).

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