

PHYTOCHEMICAL STUDY OF MURRAYA EXOTICA L. (RUTACEAE)  
I-METHOXYLATED FLAVONOIDS OF THE LEAVES :

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ABSTRACT

From the air-dried powdered leaves of *Murraya exotica* L. (Rutaceae), six methoxylated flavones were isolated and identified as: 3,5,6,7,8,3̄,4̄,5̄, -octamethoxyflavone; 3,5,6,7,3̄,4̄,5̄, -heptamethoxyflavone; 5,6,7,3̄,4̄,5̄, -hexamethoxyflavone; 5,6,7,3̄,4̄, -pentamethoxyflavone; 3,5,7,3̄,4̄,5̄ -hexamethoxyflavone and 3,5,7,8,3̄,4̄,5̄ -heptamethoxyflavone.

The identification was based on physical, chemical and spectral studies including <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS analyses.

The antimicrobial and cytotoxic activities of the isolated compounds were studied.

INTRODUCTION

*Murraya exotica* L. (*M. paniculata* Jack)<sup>1</sup> is a shrub up to 4 meters high. It is native to South east Asia<sup>2</sup> but cultivated in the Botanic Island in Aswan.

The plant is scheduled as one of the several species of family Rutaceae used successfully as anticancer drug<sup>3</sup>. Several *Murraya* species have condensed folk uses as tonic,

stomachic, stimulant, carminative and for curing many diseases<sup>4,5</sup>.

Screening for the active principles in the different organs of the plant showed the presence of flavonoidal components in the leaves.

Reviewing the current literature, **carotenoids**<sup>6</sup>, methoxylated flavones<sup>7-11</sup>, coumarins<sup>12-18</sup> and alkaloids<sup>(19-23)</sup> were isolated from Murraya exotica L. Moreover, the volatile oil of the plant was studied<sup>24</sup>.

Concerning Murraya exotica L. growing in Egypt, nothing could be traced. Therefore, it is of deamed interest to study the different constituents of it in order to evaluate its medicinal potentiality.

## EXPERIMENTAL

### General Experimental Procedures :

Melting points were uncorrected. <sup>1</sup>H NMR spectra were carried out in CDCl<sub>3</sub> at 90 MHz and chemical shifts are given in values. <sup>13</sup>C NMR spectral analyses were carried out in DMSO or CDCl<sub>3</sub> using TMS as internal standard. Column chromatography : silica gel (E.Merck) or neutral alumina (Merck). TLC : silica gel G254E, Merck) and the solvent systems :

Solvent I : pet. ether-ethyl acetate (1:1)

Solvent II : cyclohexane-ethyl acetate (1:2)

Solvent III: benzene-ethyl acetate (1:1)

Solvent IV : chloroform

Solvent V : ethyl acetate

Solvent VI : chloroform-methanol (95:5)

Solvent VII: benzene-acetone (3:4)

Solvent VIII: chloroform-methanol (97:3)

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Plant Material :

The leaves of *Murraya exotica* L. were collected in October 1984 from plants growing in the Botanic Island in Aswan. The leaves were air-dried, reduced to No.40 powder and kept in well-closed dark containers.

Extraction :

The concentrated methanolic extract of 3 kg. of the air-dried powdered leaves was successively extracted with cyclohexane, carbontetrachloride, methylenechloride, ethyl acetate and n-butanol. The concentrated carbontetrachloride extract was tested for flavonoids and showed positive results.

The carbontetrachloride fraction residue (18 g) was chromatographed over silica gel column using cyclohexane-acetone (2:1) as eluent. Fractions (25 ml each) were collected and subjected to TLC using solvent systems (I-VIII). Purification and complete isolation of the components were accomplished through preparative TLC or rechromatography over smaller columns.

Six compounds were isolated, their physical and chromatographic characters are given in Table (1).  $^1\text{H}$  NMR spectral analyses are shown in Table (2).  $^{13}\text{C}$  NMR spectral analyses are shown in Table (3).

UV and MS spectra of the isolated compounds:

COMPOUND 1 :

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log $\epsilon$ ) nm : 341 (4.22), 310(4.22), 277(4.16), 266(4.25), 252(4.19) and 209(4.73), no bathochromic shift on addition of alkali solutions.

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MS :  $M^+$  at m/z 462 (63), 447(100), 461 (10), 432(3), 341 (8), 419(2), 417(7), 389(9), 225(3), 195 (S).

COMPOUND 2 :

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log $\epsilon$ ) nm : 325(3.27), 280(2.60), 266(3.03), 240(3.03) 233(3.06), and 214(3.36), no bathochromic shift on addition of alkali solutions .

MS:  $M^+$  at m/z 432(65), 417(100), 431(14), 402(3), 401 (9), 389 (2), 387(6), 374(3), 359(8), and 195(6).

COMPOUND 3 :

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log $\epsilon$ ) nm : 322(4.03), 280 sh.(3.71), 270 (3.75), 254 sh. (3.75), 238(3.97), 230 sh. (4.01), 216(4.32) and 210 (4.28), not affected by alkali solutions.

MS :  $M^+$  at m/z 402 (28), 387 (100), 372(1), 371 (8), 357 (7), 195 (9) and 167 (27).

COMPOUND 4 :

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log $\epsilon$ ) nm : 327 (4.03), 280 sh. (3.68), 268 (3.77), 241 (3.96), 230 sh. (3.98), 214 (4.27), and 207(4.30), not affected by alkali solutions.

MS :  $M^+$  at m/z 372 (23), 357(100), 341 (17), 195 (3), 167 (17), 165 (5), and 139 (3).

COMPOUND 5 :

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log $\epsilon$ ) nm : 325(4.14), 277 (3.92), 270 sh. (4.00), 260 (4.04), 254 sh. (4.30), 240 sh. (4.10) and 208 (4.59); not affected by alkali solutions.

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MS :  $M^+$  at m/z 402 (97), 387 (100), 401 (30), 372 (4), 371 (20), 359 (5), 195 (6), 181 (16), 152 (5) and 151 (23) .

COMPOUND 6 :

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  : Log( $\epsilon$ ) nm : 348 (4.22), 310 sh. (4.16), 274 (4.27), 266 sh. (4.25) and 209 (4.73); not affected by alkali solutions.

MS :  $M^+$  at m/z 432 (82), 417 (100), 431 (23), 401 (16), 195 (4) and 167 (6) .

RESULTS AND DISCUSSIONS

The carbontetrachloride fraction of the methanolic extract of the leaves of *Murraya exotica* L. gave positive results for presence of flavonoids, when chromatographed over silica gel column, six flavonoidal components were obtained (1-6) .

COMPOUND 1 ;  $C_{23}H_{26}O_{10}$ , m.p.  $157^{\circ}C$ , it gives no colour with ferric chloride reagent indicating the absence of phenolic OH groups.  $^1H$  NMR spectrum (Table 2) showed signals for the unsubstituted C-2' and C-6' at 7.52 ppm<sup>9</sup> . The presence of eight  $CH_3O$ - groups indicated by the signals appearing between 3.91 ppm and 4.10 ppm.

The fragmentation pattern of this compound shows a base peak at m/z 447. Further loss of  $CH_3$  group gives peaks at m/z 432 and 417. The peaks at m/z 225 and 195 indicate that ring A have four  $CH_3O$ -groups while ring B have three  $CH_3O$ -groups.

The correct structure of the isolated compound was established by its  $^{13}C$  NMR spectrum (Table 3). The  $CH_3O$ -groups are located at C-3, C-5, C-6, C-7, C-8, C-3', C-4', C-5' 26-28 .

COMPOUND 1 , is identical with the previously isolated myricetin hexamethyl ether<sup>(9)</sup>. However, the study of its <sup>13</sup>C NMR is reported here for the first time confirming its identity.

COMPOUND 2, C<sub>22</sub>H<sub>24</sub>O<sub>9</sub>, m.p. 155°C, it had no free OH groups. <sup>1</sup>H NMR spectrum (Table 2) shows signals for the unsubstituted C-2' and C-6' (7.35 ppm) and C-8 (6.74 ppm). The presence of seven CH<sub>3</sub>O-groups is indicated by the signals between 3.88 ppm and 4.00 ppm.

The fragmentation pattern of compound-2 shows a base peak at m/z 417 (M<sup>+</sup>-CH<sub>3</sub>). Further loss of CH<sub>3</sub> group gives peaks at m/z 402 and 387. The peak at m/z 195 shows that both ring A and ring B have three CH<sub>3</sub>O-groups.

The correct structure of compound-2 was established by its <sup>13</sup>C NMR spectrum. The CH<sub>3</sub>O-groups are located at C-3, C-5, C-6, C-7, C-3', C-4', and C-5'.<sup>27-30</sup>

Compound 2 is identical with that previously isolated from M. exotica L. by Davis L. Dreyer<sup>12</sup>.

However, the study of <sup>13</sup>C NMR is reported here for the first time confirming its identity.

COMPOUND 3 ; C<sub>21</sub>H<sub>22</sub>O<sub>8</sub>, m.p. 115°C, M<sup>+</sup> = 402, it gives no colour with ferric chloride reagent indicating the absence of hydroxyl groups.

<sup>1</sup>H NMR spectrum (Table 2) shows a signal at 7.08 ppm which is similar to that of compounds 1 and 2; this means that no substitution at C-2' and C-6'. The appearance of two signals at 6.8 and 6.6 ppm is correlated to the free protons of C-8 and C-3 respectively. The presence of six methoxy group is indicated by the signals at 3.92 (6-H), 3.96 (6-H) and 4.00 ppm (6-H).

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The fragmentation pattern of compound-3 shows a base peak at  $m/z$  387 ( $M^+ - CH_3$ ). Further loss of  $CH_3$  group gives peaks at  $m/z$  372 and 357. The appearance of peaks at 195 and 167 proves that both ring A and ring B have three  $CH_3O$ -groups.

The final structure of compound 3 was confirmed by its  $^{13}C$  NMR spectrum (Table 3). The  $CH_3O$ -groups are located at C-5, C-6, C-7, C-3', C-4', and C-5' <sup>27-30</sup>.

Compound-3 is identical with that isolated from Bauhinia Championii Benth <sup>31,32</sup>. However, this is the first report of this compound in family Rutaceae.

Compound 4 ;  $C_{20}H_{20}O_7$ , m.p. 178°C, has no free OH groups.

$^1H$  NMR study of this compound (Table 2) shows signals at 6.80 and 6.60 ppm which indicated that both C-8 and C-3 have no substituents. At 7.98 ppm (1H, dd), 7.68 ppm (1H, d) and 7.37 ppm (1H, d) indicating that the free protons may be at C-6', C-2' and C-5'.

MS of compound-4 shows a base peak at  $m/z$  357 ( $M^+ - CH_3$ ). The appearance of peaks at  $m/z$  195 and 165 indicates that ring A has three  $CH_3O$ -groups while ring B has only two groups.

$^{13}C$  NMR of compound-4 (Table 3) confirms the given structure and shows that the positions of  $CH_3O$ -groups are at C-5, C-6, C-7, C-3' and C-4' <sup>30</sup>.

Compound 4 is identical with that isolated from Veronica filiformis <sup>30</sup> and from Chromolaena odorata <sup>34</sup>. The compound was also prepared from the correlated penta-hydroxyflavone <sup>35</sup>.

However, this is the first report of this compound in family Rutaceae.

COMPOUND 5,  $C_{21}H_{22}O_8$ , m.p.  $154^{\circ}C$ , it gives no colour with ferric chloride reagent indicating the absence of free OH groups.

$^1H$  NMR spectrum (Table 2) shows two signals, each is doublet at 6.35 and 6.44 ppm for C-6 and C-8 protons. Another signal at 7.37 ppm which is correlated to both C-2' and C-6' protons. The presence of six  $CH_3O$ -groups is indicated by the signals appearing between 3.89 and 3.97 ppm.

The fragmentation pattern of compound 5 shows a base peak at  $m/z$  387 ( $M^+ - CH_3$ ). The two characteristic peaks at  $m/z$  181 and 195 means that ring A has only two  $CH_3O$ -groups while ring B has three  $CH_3O$ -groups.

$^{13}C$  NMR of the isolated compound (Table 3) confirms the proposed structure and shows that the  $CH_3O$ -groups are located at C-3, C-5, C-7, C-3', C-4', and C-5'.<sup>36</sup>

Compound 5 is identical with that isolated from Soymida febrifuga<sup>(37)</sup> and this is the first report of this compound in family Rutaceae.

COMPOUND 6,  $C_{22}H_{24}O_9$ , m.p.  $189-190^{\circ}C$ , it has no free OH groups.

$^1H$  NMR spectrum of compound 6 (Table 2) shows a signal at 7.54 ppm of two protons of C-2' and C-6'<sup>9</sup>. Another signal appears at 6.42 ppm which is correlated to the protons of C-6. The presence of the seven  $CH_3O$ -groups is indicated by the signals appearing between 3.91 and 4.00 ppm.

MS of compound 6 shows a base peak at  $m/z$  417 ( $M^+ - CH_3$ ). The characteristic peak at  $m/z$  195 shows that both ring A and ring B have three  $CH_3O$ -groups according to the fragmentation pattern of the isolated compound.



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$^{13}\text{C}$  NMR spectral analysis of compound 6 (Table 3) shows that  $\text{CH}_3\text{O}$ -groups are located at C-3, C-5, C-7, C-8, C-3, C-4 and C-5 while C-6, C-2 and C-6 have no oxygen function<sup>31-35</sup>.

Compound 6 was found to be identical with hisbiscetin heptamethyl ether which was isolated from *Murraya exotica* L. However,  $^{13}\text{C}$  NMR and MS assignments which were done for the first time confirmed the identity.

CYTOTOXIC ACTIVITY OF THE ISOLATED COMPOUNDS :

In-vitro cytotoxic tests were carried out on the isolated components. The effects of these compounds against  $\text{P}_{388}$  and KB cells (Table 4) shows that compound I has an inhibitory effect against  $\text{P}_{388}$  cells and a moderate effect against KB cells. Compound 3 showed a moderate effect against  $\text{P}_{388}$ . The other compounds showed no effect on both systems.

ANTIMICROBIAL ACTIVITY OF THE ISOLATED COMPOUNDS :

The antimicrobial activities of the isolated compounds were studied (Table 5). Both antibacterial and antifungal properties were determined using four species of fungi and one species of bacteria (*Bacterium subtilis*) by the usual agar-cup method<sup>39</sup>. The isolated compounds show no effect on *Bacterium subtilis*, but compound 3 shows inhibitory effect on *Coprinus cinereus*, *sparolegnia* and *Botryis cinerea* and compound 1 shows a moderate effect against *Copriions cinereus*. The other isolated compounds show slight or no effect.

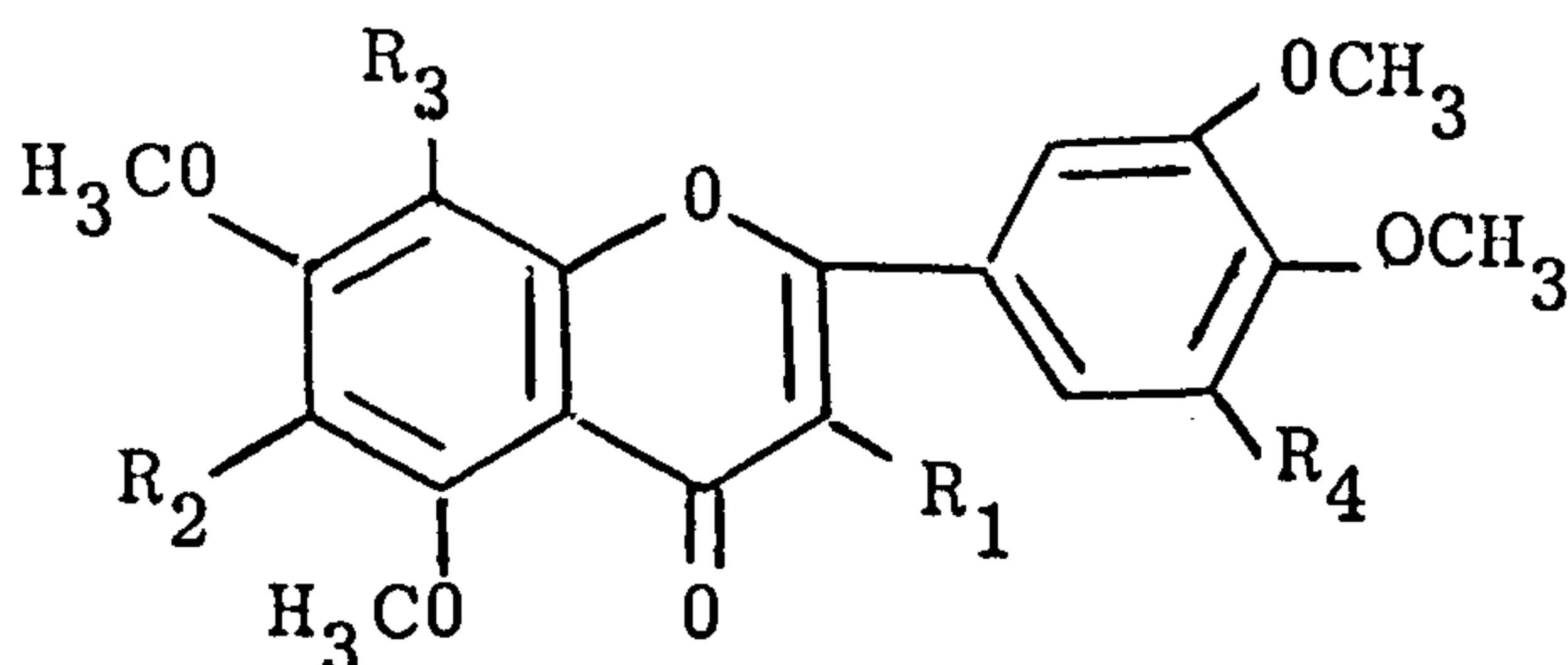
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Table I : Characters of the Isolated Compounds :

No.	R <sub>f</sub>	Solvent system	colour		crystal form m.p.
			UV	anisaldehyde H <sub>2</sub> SO <sub>4</sub> reagent	
1	0.25	I	y	y	yellowish-white needles (20 mg)
	0.28	II			
2	0.28	III	y	y	yellowish-white needles (37 mg)
	0.47	V			
	0.64	IV			
3	0.42	v	y	y	yellowish-white needles (17 mg)
	0.50	vi			
	0.25	III			
4	0.38	V	y	y	yellowish-white needles (9.9 mg)
	0.42	VI			
	0.22	III			
5	0.20	III	y	y	yellowish-white needles (10 mg)
	0.39	VI			
	0.30				
6	0.29	VII	y	y	yellowish-white needles (7.8 mg)
	0.46	VIII			

y : yellow colour.



- Compound-1: R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> = OCH<sub>3</sub>  
 Compound-2: R<sub>1</sub>, R<sub>2</sub>, R<sub>4</sub> = OCH<sub>3</sub>, R<sub>3</sub> = H  
 Compound-3: R<sub>2</sub>, R<sub>4</sub> = OCH<sub>3</sub>, R<sub>1</sub>, R<sub>3</sub> = H  
 Compound-4: R<sub>2</sub> = OCH<sub>3</sub>, R<sub>1</sub>, R<sub>3</sub>, R<sub>4</sub> = H  
 Compound-5: R<sub>1</sub>, R<sub>4</sub> = OCH<sub>3</sub>, R<sub>2</sub>, R<sub>3</sub> = H  
 Compound-6: R<sub>1</sub>, R<sub>3</sub>, R<sub>4</sub> = OCH<sub>3</sub>, R<sub>2</sub> = H

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Table 2 : <sup>1</sup>H-NMR data (90 MHz) of the isolated compounds

	1	2	3	4	5	6
H-3	---	---	6.60(S) (1H)	6.60(S) (1H)	---	---
H-6	---	---	---	---	6.35(d) (J=2.5(Hz) (1H)	6.42(S)(1H)
H-8	---	6.74(S) (1H)	6.80(S) (1H)	6.8 (S) (1H)	6.44(d) (J=2.5(Hz) (1H)	-----
H-2	7.52(S) (2H)	7.35(S) (2H)	7.08(S) (2H)	7.68(d) (J=2Hz) (1H)	7.37(S) (2H)	7.54(S)(2H)
H-5	---	---	---	7.37(d) (J=9Hz) (1H)	---	-----
H-6	7.52(S) (2H)	7.35 (S) (2H)	7.08 (S) (2H)	7.98(dd) (J=9 Hz, J=2Hz) (1H)	7.37 (S) (2H)	7.54(S)(2H)
0 OH <sub>3</sub>	3.91(S)(3H)	3.88(S)(3H)	3.92(S)	3.94(m) (15H)	3.89(S)(3H)	3.91(S)(6H)
	3.95(S)(12H)	3.92(S)(3H)	(6H)		3.91(S)(3H)	3.94(S)(9H)
	3.98(S)(3H)	3.94(S)(9H)	3.96 (S) (6H)		3.93(S)(3H)	4.0 (S)(6H)
	4.0(S)(3H)	3.98(S)(3H)	(6H)		3.94(S)(6H)	
	4.10(S)(3H)	4.0 (S)(3H)	4.00(S) (6H)		3.97(S)(3H)	

Table 3 :  $^{13}\text{C}$  NMR Spectral Analysis of the Isolated Compounds,  
 $\text{CDCl}_3$ , TMS as Internal Standard.

Carbon atom.	Chemical shift, ppm					
	comp.1*	comp.2	comp.3	comp.4	comp.5	comp.6
C-2	151.7	152.6	158.0	160.9	157.8	152.6
C-3	143.2	140.6	108.0	106.8	140.6	152.6
C-4	172.2	173.6	176.7	176.7	171.4	175.5
C-5	150.8	153.0	153.1	152.3	161.2	153.5
C-6	139.0	140.3	139.0	140.4	96.0	96.7
C-7	146.8	157.9	161.1	156.0	164.0	154.3
C-8	137.2	96.1	96.7	96.3	92.6	127.9
C-9	147.2	153.6	154.9	154.1	152.6	146.9
C-10	114.2	113.0	113.5	108.9	109.1	104.7
C-1'	125.0	126.0	127.2	123.8	126.0	126.0
C-2'	105.4	106.4	105.4	111.5	106.3	106.4
C-3'	152.7	153.2	154.0	149.5	153.3	153.9
C-4'	140.4	141.3	140.0	151.0	141.7	141.3
C-5'	152.7	153.2	154.0	112.7	153.1	153.9
C-6'	105.4	106.4	105.4	102.7	106.3	106.4
OCH <sub>3</sub> at :						
C-3	59.3	60.1	---	---	56.4	56.2
C-5	61.7	62.2	62.0	61.9	60.5	60.7
C-6	61.7	61.5	59.6	61.4	---	---
C-7	61.7	56.3	56.4	55.9	55.9	56.0
C-8	61.7	---	---	---	---	59.9
C-3'	55.9	56.5	56.3	55.5	56.4	55.8
C-4'	60.0	61.0	60.8	55.5	60.0	60.3
C-5'	55.9	56.5	56.9	---	56.4	55.8

\* DMSO instead of  $\text{CDCl}_3$

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Table 4: Cytotoxic Activities of the Isolated Compounds of  
M. exotica L.

Compound	LD <sub>50</sub> (ug/ml) "P 388 "	LD <sub>50</sub> (ug/ml) "KB" cells
(1)	4.5	8.8
(2)	12.0	>50.0
(3)	9.0	18.9
(4)	11.1	14.7
(5)	12.7	>50.0
(6)	32.1	>50.0

Table 5: Antifungal Activities of the Isolated Compounds  
of M. exotica L.

Compound	Coprinus cinereus	Botrytis cinerea	Rhizoctonia salani	Sparolegnia
	24 hours	24 hours	24 hours	24 hours
(1)	8	0	0	(+)
(2)	(+)	0	5	(+)
(3)	15	10	(+)	15
(4)	0	(+)	(+)	0
(5)	0	0	0	0

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الدراسة الكيمائية للمحتوى الفلافونويدى

لنبات المورايا اكسوتيكال

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قام الباحثون باستخلاص أوراق نبات المورايا أكسوتيكال بالكحول الميثيلى ثم تجزئة الخلاصة الكحولية المركزه بالهكسان الحلقى ، رابع كلوريد الكربون ، كلوريد الميثيلين ، خلاص الايثيل والبيوتانول حيث تم فصل ستة مركبات فلافونيدية من خلاصة رابع كلوريد الكربون وتم التعرف عليها باستخدام الطرق الفيزيائية والكيمائية والطيفية مثل الرنين النووى المغناطيسى البروتونى والكربونى وكذلك مطياف الكتله على النحو التالى :-

٣، ٥، ٦، ٧، ٨، ٣، ٤، ٥ - ثمانى ميثوكسى فلافون  
 ٥، ٦، ٧، ٣، ٤، ٥ - سداسى ميثوكسى فلافون  
 ٣، ٥، ٦، ٧، ٣، ٤، ٥ - سباعى ميثوكسى فلافون  
 ٥، ٦، ٧، ٣، ٤ - خماسى ميثوكسى فلافون  
 ٣، ٥، ٦، ٧، ٣، ٤، ٥ - سداسى ميثوكسى فلافون  
 ٣، ٥، ٦، ٧، ٨، ٣، ٤، ٥ - سباعى ميثوكسى فلافون

وتمت دراسة التأثير البيولوجى للمركبات التى تم فصلها بدراسة تأثير كل منها على أنواع خاصة من البكتريا والفطريات وكذلك دراسة التأثير القاتل على نوعين من خلايا السرطان وقد أظهرت الدراسة تأثيرا ايجابيا لبعض منها .

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