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Original Article

Isolation and Characterization of Flavones from *Artemisia monosperma*

Lubna Abu-Niaaj*, Ibrahim Katampe

ABSTRACT

Background: *Artemisia monosperma* (Delile) is a green aromatic shrub that grows widely in the deserts of Middle East, Africa and China. This plant is commonly used in folk medicine as a remedy of a wide range of illness including gastrointestinal disorders, inflammation, diabetes and microbial infection. The different categories of the secondary metabolites identified from *Artemisia* species are recognized for their biological activities as antioxidants, anti-inflammatory and antimicrobial compounds. **Objective:** This study aims to isolate new flavonoids from *A. monosperma* that might have potential biological activities thus be translated into pharmaceutical uses. **Materials and Methods:** Air-dried *A. monosperma* extraction was done using different organic solvents. The methanolic extract was selected for isolation of flavonoids using column chromatography and thin layer chromatography. The chemical structures of the isolated flavones determined based on spectroscopic analysis of ultraviolet, mass and nuclear magnetic resonance spectra. **Results:** Nine flavone aglycones were isolated and identified from the methanolic extract; four of which are reported for the first time from *A. monosperma*. These include: 5-Hydroxy-3',4', 6,7-tetramethoxyflavone; 3',5-dihydroxy-4',6,7-trimethoxyflavone (eupatorin); 5,6-dihydroxy-4',7-dimethoxyflavone(ladanein); and 2',4',5-trihydroxy-5',6,7–trimethoxyflavone (arcapillin). The remaining five flavones were previously identified from this plant as : 4',5-Dihydroxy-3',6,7-tri-methoxyflavone(cirsilineol);5,7-dihydroxy-3',4',6-trimethoxy-flavone (eupatilin);4',5,7-trihydroxy-3',6-dimethoxyflavone(jaceosidin);4',5-dihydroxy-6,7-dimethoxy-flavone (circimaritin) and 4',5,7-trihydroxy-6-methoxyflavone (hispidulin). In addition, two acetophenone derivatives were isolated from fractions yielded selected flavones and these were identified as 4-hydroxyacetophenone and 3-(2-hydroxymethyl-2-buten-4-yl)-4-hydroxy-aceto-phenone. **Conclusion:** This successful isolation of these natural flavonoids from *A. monosperma* can contribute further to the evaluation of bioactive compounds against disorders including but not limited to inflammatory associated disorders and microbial infections.

Key words: *Artemisia monosperma,* Flavones, Antioxidants, Antimicrobial activity,

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INTRODUCTION

Artemisia monosperma (Delile) is a green aromatic perennial shrub that belongs to the family Asteraceae (Compositae) and it grows widely in the deserts of Middle East, Africa and China.[1-3] The plant is reputed in folk medicine for treatment of gastrointestinal disorders,[4] diabetes,[5] rheumatic pain, fever and to induce abortion.[6] In addition to the high antioxidant activity,[7] *A. monosperma* is reported for antimicrobial activities[8-9] as well as its insecticidal and antimalarial potentiation.[10-13] Grech-Baran and Pietrosiuk[14] reported the synthesis of two drugs, Artemisinin and Arglabin, first isolated from *Artemisia* species.[15] Artemisinin derivatives have become standard treatment worldwide against malaria while Arglabin and its derivatives have shown antitumor activity against multiple tumor cell lines.[16] The wide-range of biological activities of *A. monosperma* is due to its content of a variety of secondary metabolites such as flavonoids,[17-20] alkaloids,[9] coumarins,[21-22] acetylenic compounds,[2,13] oils,[1,23-32] and terpenoids.[33] This study aims to isolate new flavonoids from *Artemisia monosperma* of potential pharmaceutical activities.

MATERIALS AND METHODS

Collection of Plant

Artemisia monosperma (Delile) was collected from the Southern desert of Jordan near Irwaishid. The voucher specimen was authenticated by professor Dawud El-Eisawi, a botanist of the University of Jordan and it was deposited as "S. Abdalla #251", in the herbarium of University of Jordan, Amman, Jordan. The collected plant was air dried away from the direct sunlight.

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Extraction of Plant

Several organic solvents were used to extract flavonoids from the plant. The dried plant (7.0 Kg) was immersed in petroleum ether at room temperature for 10 days with frequent agitation to extract lipids. The petroleum ether was filtered, evaporated under reduced pressure to give a crude extract that was kept for later use. The plant material was further extracted repeatedly with multiple organic solvent systems as shown in Figure 1. All chemicals and organic solvents used were of analytical grade and purchased from Fisher Scientific.

Fractionation of the Methanolic Crude Extract

The methanolic crude extract (183 g) was subjected to fractionation using column chromatography and Thin Layer Chromatography (TLC). It was adsorbed on 150g silica gel, and loaded on a previously packed silica gel column with chloroform (8cm x 60cm, 1kg silica gel 60 PF254, 70-230 mesh). Elution of the column was initially with chloroform then the polarity was increased gradually by adding 95% ethanol and /or methanol. Eluents collected in 500 ml, analyzed by TLC, and combined into twelve fractions that were concentrated and subjected to further fractionation by column chromatography.

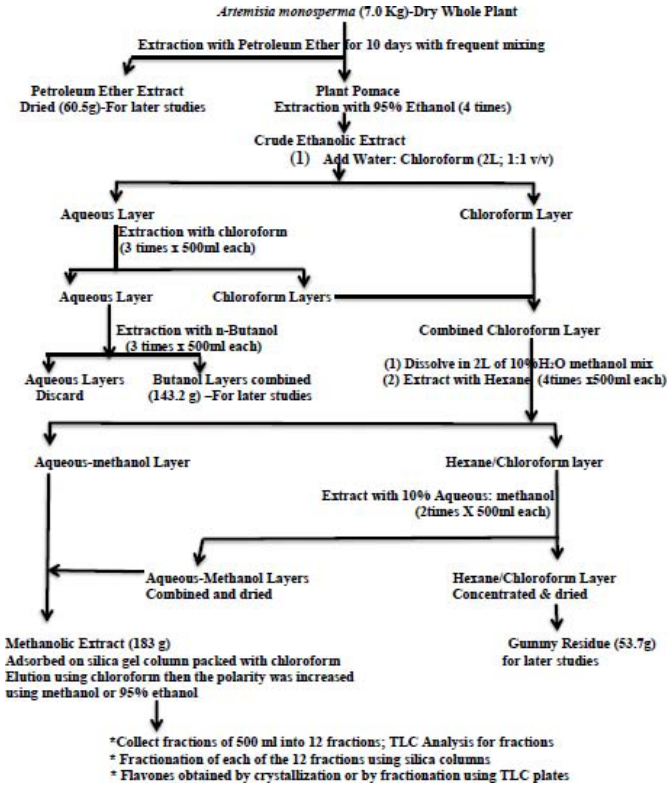


Figure 1: Isolation of Different Flavones from *Artemisia monosperma* (Delile)

Purification

The identified flavones were isolated from eight fractions subjected to column chromatography. Each selected fraction was loaded on a silica column (0.35-0.50 Kg silica gel 60 PF254) packed in benzene: ethyl acetate (85%-75%). The loaded quantities were: fraction II (10.7g); fraction III (8.0g of 14.2g); fraction IV (11.7 g); fraction V (15g of 25 g), fraction VI (8.0g); fraction VII (7.5g); VIII (7.5g). The polarity of the eluted solvent was increased gradually until pure ethyl acetate was reached. Fractions were collected in 500ml and combined according to their TLC analysis. Further purification of fractions was achieved by either crystallization or by fractionation by TLC plates using suitable solvent systems. The structural elucidation of compounds was achieved by spectral analysis of ^{1}H-NMR, UV, and mass data.

Ultraviolet (UV) Analysis

UV spectra were recorded on a Nikon 810 spectrophotometer. Identification of functional groups on the flavone nucleus was obtained by comparison of the spectrum of the compound in methanol to those obtained in the presence of acidic or basic shift reagents (sodium methoxide, aluminum chloride in presence or absence of hydrochloric acid).

Mass Spectroscopy Analysis

The mass spectral data obtained by the electron impact method (eim) at 70-90 eV on a kratos MS 9/50 double-focusing high resolution mass spectrophotometer.

Nuclear Magnetic Resonance (NMR) Spectroscopic Analysis

The proton nuclear magnetic resonance (200 MHz ^{1}H-NMR) spectra were recorded on a Brucker WP-200 spectrometer. Proton chemical shifts presented as parts per million (ppm) on a scale relative to tetramethylsilane using a deuterated chloroform peak at 7.26 ppm as an internal standard. Low-soluble compounds were dissolved in hexadeuteriodimethyl sulfoxide (DMSO-d_6). The coupling constants are given in Hertz (Hz).

RESULTS

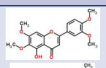
Four flavone aglycones are reported for the first time from *A. monosperma*. These are: 5-hydroxy-6,7, 3?,4?-tetramethoxyflavone, ladanein, eupatorin and arcapillin. Other five previously reported flavone aglycones are identified as: eupatilin, circimaritin, cirsilineol, hispidulin, and jaceosidin. Table 1 lists the obtained flavones, their chemical formula, common names, quantities, and molecular weights. The structural elucidation of compounds was achieved by spectral analysis of proton nuclear magnetic resonance, ultraviolet, and mass data. Table 2 lists the analysis of UV spectra, and Figure 2 shows the ^{1}H-NMR spectra. The organic solvent mixtures used to obtain the flavones by crystallization or by fractionation using TLC plates as listed in Table 3.

Two acetophenone derivatives were also isolated and identified as 4-hydroxyacetophenone (Figure 3) and 3-(2- Hydroxymethyl-2-buten-4-yl)-4-Hydroxyacetophenone (Figure 4) from fractions previously yielded flavones. The TLC of fraction IV using chloroform: methanol: ammonia gas (8:2 v/v) produced 115 mg of 4-Hydroxyacetophenone which is known as piceol. 500 mg of 3-(2- Hydroxymethyl-2-buten-4-yl)-4- hydroxyacetophenone was obtained by TLC of fractions V and VII using chloroform: methanol: ammonia gas (7.5: 2.5 v/v). The UV spectrum of this compound in methanol showed a band at 278 which was shifted to 354 upon addition of sodium methoxide indicating the presence of a phenolic group. The mass spectrum showed a molecular ion peak at m/e 202 due to the loss of a water molecule.

DISCUSSION

Flavones, a subgroup of flavonoids, have a basic skeleton that consists of three rings as shown below. The antioxidant activity of flavonoids is

Table 1: Flavones isolated from *Artemisia monosperma* (Delile); chemical names and structures, common names, obtained quantities and molecular formula

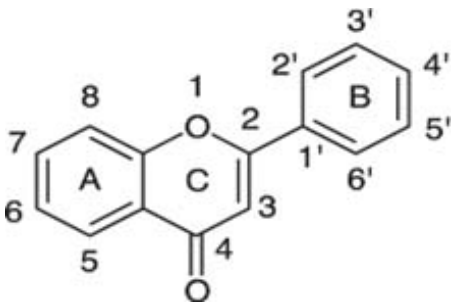
	Chemical Name of Compounds	Chemical Structure	Common Name	Quantity (mg)	Mr (g/mol)
1	5-Hydroxy-3',4',6,7-tetramethoxyflavone		*N/A	175	358
2	3',5-Dihydroxy-4',6,7-trimethoxyflavone		*Eupatorin	70	344
3	5,6-Dihydroxy-4',7-dimethoxyflavone		*Ladanein	55	314
4	5,7-Dihydroxy-3',4',6-trimethoxyflavone		Eupatilin	640	344
5	4',5-Dihydroxy-3',6,7-trimethoxyflavone		Cirsilineol	280	344
6	4',5,7-Trihydroxy-3',6-dimethoxyflavone		Jaceosidin	60	330
7	4',5-Dihydroxy-6,7-dimethoxyflavone		Cirsimaritin	415	314
8	4',5,7-Trihydroxy-6-methoxyflavone		Hispidulin	20	300
9	2',4',5-Trihydroxy-5',6,7-trimethoxyflavone		*Arcapillin	180	360

* Indicates flavones isolated for the first time from *A. monosperma.*

Table 2: UV spectra for the Isolated Flavones from *Artemisia monosperma.*

	Chemical Name of Compounds	Common Name	CH_3OH	$NaOCH_3$	$AlCl_3$	$ALCl_3$+HCl
1	5-Hydroxy-3',4', 6,7-tetramethoxyflavone	N/A	340, 278	340, 288	370, 286	360, 289
2	3',5-Dihydroxy-4', 6,7-trimethoxyflavone	Eupatorin	338, 276	368, 310, 276	368, 284	354, 290
3	5,6-Dihydroxy-4',7-dimethoxyflavone	Ladanein	330,274	368, 296sh, 274	354,303sh, 280sh	350, 300
4	5,7-Dihydroxy-3',4',6-trimethoxyflavone	Eupatilin	342,276	370,310, 276	370,282, 260	358,290, 254
5	4',5-Dihydroxy-3',6,7-trimethoxyflavone	Cirsilineol	342,276, 242	406,304sh, 266	376,286	364,286
6	4',5,7-Trihydroxy-3',6-dimethoxyflavone	Jaceosidin	344,274	408,338, 258sh	378, 281sh, 261	364,286
7	4',5-Dihydroxy-6,7-dimethoxyflavone	Cirsimaritin	332,278	389,296sh, 272	362,302, 290sh	254,300, 262sh
8	4',5,7, -Trihydroxy-6-methoxyflavone	Hispidulin	334,276	394,326	362,302	352, 300
9	2',4',5-Trihydroxy-5', 6,7- rimethoxyflavone	Arcapillin	268, 288sh	422, 322sh, 288sh	406, 316, 276	398, 316sh, 278

related to their structure especially the hydroxy substitution of the aromatic A and B rings and the substitution pattern of the C-ring.[34]



We report the isolation and characterization of nine flavones from the methanolic extract of *A. monosperma;* four of which are reported for the first time from this plant. The five previously reported flavones from *Artemisia monosperma* are eupatilin,[17] circimaritin, cirsilineol, jaceosidin and hispidulin.[20] The data of ^{1}H-NMR, UV and mass obtained for the different flavones match those reported in the literature.[20,34-41] The two acetophenone derivatives are of importance because of the potential of their anti -oxidative and anti-inflammatory effects of acetophenones.[38]

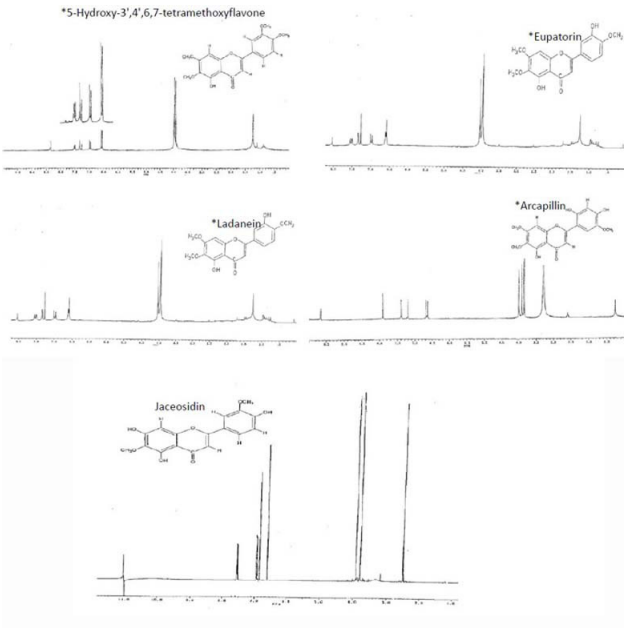


Figure 2: The 200 MHz ^{1}H-NMR spectra of isolated flavones from *A. monosperma.*

Table 3: Purification of flavones using different organic solvent systems.

	Chemical Name of Compounds	Common Name	Fraction	Solvent Used (v/v)
1	5-Hydroxy-3',4',6,7-tetramethoxyflavone	N/A	II	Crystallization from Methanol
2	3',5-Dihydroxy-4',6,7-trimethoxyflavone	Eupatorin	II	EtAc: Pet. (3:7)
3	5,6-Dihydroxy-4',7-dimethoxyflavone	Ladanein	II	Ben: $CHCl_3$: MeOH (3:6:1) & EtAc: Ben (2:8)
4	5,7-Dihydroxy-3',4',6-trimethoxyflavone	Eupatilin	II&III	Ben: $CHCl_3$: MeOH (3:6:1)
5	4',5-Dihydroxy-3',6,7-trimethoxyflavone	Cirsilineol	II&III	EtAc: Ben. (3:7)/twice
6	4',5,7-Trihydroxy-3',6-dimethoxyflavone	Jaceosidin	IV	Methanol and/or cyclohex: Ac.(7:3)
7	4',5-Dihydroxy-6,7-dimethoxyflavone	Cirsimaritin	V	Methanol or $CHCl_3$: MeOH : NH_3 (g) (8:2)
8	4',5,7, -Trihydroxy-6-methoxyflavone	Hispidulin	VI	$CHCl_3$: MeOH: NH_3 (g) (8:2)
9	2',4',5-Trihydroxy-5',6,7- trimethoxyflavone	Arcapillin	VI,VII, VIII	$CHCl_3$: MeOH: NH_3 (g) (7.5:2.5)

Ben.=Benzene; $CHCl_3$=Chloroform; MeOH=Methanol; EtAc.=Ethyl acetate; Pet=Petroleum Ether; NH_3(g)= Ammonia Vapor.

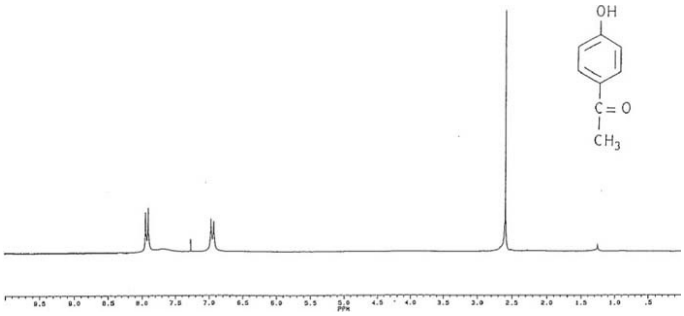


Figure 3: The ^{1}H-NMR spectrum of 4-Hydroxyacetophenone isolated from *A. monosperma.*

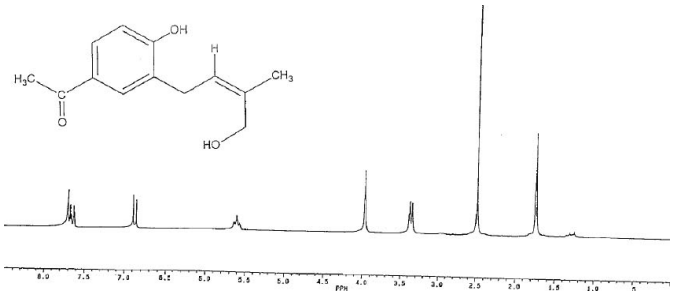


Figure 4: The ^{1}H-NMR spectrum of 3-(2- Hydroxymethyl-2-buten-4-yl)-4- hydroxyl-acetophenone isolated from *A. monosperma.*

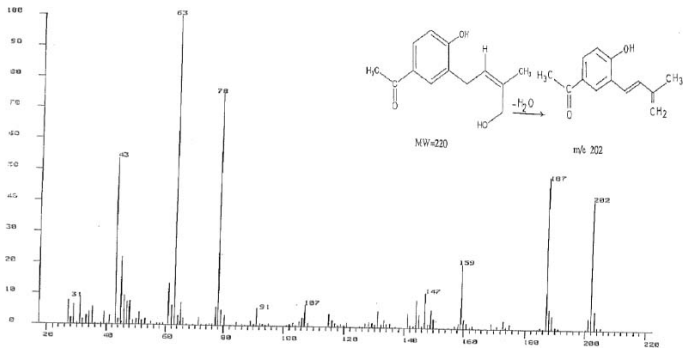


Figure 5: The mass spectra of 3-(2- Hydroxymethyl-2-buten-4-yl)-4-hydroxyl-acetophenone.

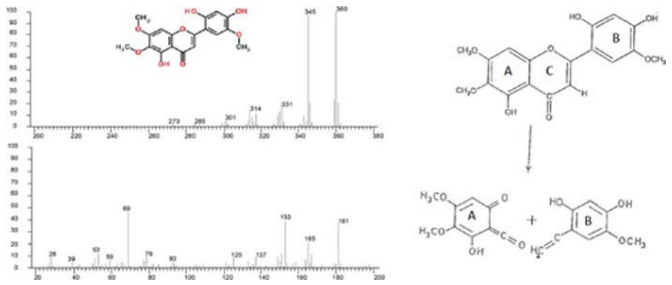


Figure 6: The mass spectrum and fragmentation pattern of Arcapillin**.**

Mass Spectra

The mass spectrum of each flavone displayed a molecular ion peak which matches its molecular formula. The fragmentation pattern was helpful in determining the substitution pattern because the resulting C-ring fragments as well as those of A and B rings are typical for each flavone.[34] For example, the structure of arcapillin was confirmed by the mass spectrum which revealed the presence of the A and B rings which is indicative of the two methoxyl groups in ring A (Figure 6). All isolated flavones showed similar fragmentation patterns.[38-41] The mass spectrum 4-hydroxyacetophenone showed a molecular ion peak at m/e 136 which corresponds to its molecular weight and the mass spectrum of 3-(2-hydroxymethyl-2-buten-4-yl)-4- hydroxyacetophenone showed a molecular ion peak at m/e 202 due to the loss of a water molecule (Figure 5).

Ultraviolet Spectroscopy

A summary for the UV spectral peaks under different conditions are listed in Table 2. The existence of a flavone nucleus is indicated by the UV spectrum of a flavone in methanol based on the appearance of two major absorption peaks; band I and band II.[39] The range of absorption for each band and subsequent bathochromic shift and change in the magnitude of peak intensity upon addition of different reagents determined the functional groups on the flavone nucleus.[34,39]

The UV spectra for the two isolated acetophenone derivatives confirmed the presence of a phenolic group. The UV spectrum of 4-Hydroxyacetophenone in methanol showed a band at 277 which was shifted to 322 upon addition of sodium bicarbonate. This shift confirmed the presence of a phenolic hydroxyl group. The UV spectrum of 3-(2- hydroxymethyl-2-buten-4-yl)-4- hydroxyacetophenone in methanol showed a band at 278 which was shifted to 354 upon addition of sodium methoxide indicating the presence of a phenolic group.

Proton Nuclear Magnetic Resonance (^{1}H-NMR)

^{1}H-NMR spectra of isolated flavones are shown in Figure 2. Analysis of spectra for all isolated compounds was similar to literature data.[40-42]

The two isolated acetophenone derivatives were confirmed by analysis of their ^{1}H-NMR spectra.

The 4-hydroxyacetophenone showed a single peak at 2.6 ppm indicating the three protons of the acetyl group (Figure 3). The aromatic ring protons appeared as two doublets at 7. 9 ppm and 6.9 ppm, a typical pattern for the para-substituted phenyl rings. Analysis of its ^{1}H-NMR spectrum (Figure 4) of 3-(2- Hydroxymethyl-2-buten-4-yl)-4- hydroxyacetophenone matches literature data.[22]

CONCLUSION

Artemisia monosperma is rich in different categories of secondary metabolites known for their diverse biological activities. Flavonoids subgroups

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

ABBREVIATIONS

NMR: Nuclear Magnetic Resonance**; UV:** Ultraviolet; **TLC:** This Layer Chromatography; **NaOCH:** $_3$Sodium methoxide; **$AlCl_3$:** Aluminum Chloride**; HCl:** Hydrochloric Acid; **ppm:** parts per million.

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SUMMARY

- *Artemisia mosopserma* methanolic extract was fractionated to obtain nine flavone aglycones.
- This study reports four flavone aglycones for the first time from *A. monosperma*; 5-Hydroxy-3',4',6,7-tetramethoxyflavone;3',5-dihydroxy-4',6,7-trimethoxyflavone(eupatorin);5,6-dihydroxy-4',7-dimethoxyflavone (ladanein); and 2',4',5-trihydroxy-5',6,7–trimethoxyflavone (arcapillin).
- This study reports five flavone glycones previously identified from *A. monosperma* and these are:4',5-Dihydroxy-3',6,7-trimethoxyflavone(cirsilineol);5,7-dihydroxy-3',4',6-trimethoxyflavone (eupatilin);4',5,7-trihydroxy-3',6-dimethoxyflavone(jaceosidin);4',5-dihydroxy-6,7-dimethoxy-flavone (circimaritin) and 4',5,7-trihydroxy-6-methoxyflavone (hispidulin).
- Two acetophenone derivatives were isolated;4-hydroxyacetophenone from the fraction yielded jaceosidin and 3-(2-hydroxymethyl-2-buten-4-yl)-4-hydroxyacetophenone from both fractions yielded circimaritin and arcapillin.

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