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Chemical constituents and bioactivities of *Malabaila suaveolens*

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ABSTRACT

Volatile oil components, fatty acids, β-amyrin, and sterols were identified in the *n*-hexane extract of *Malabaila suaveolens* Coss. fruits. Angelicin, 4,7,9-trimethyl psoralen, isopimpinellin and umbelliferone were isolated from the dichloromethane extract of the plant. 5-Hydroxy 7, 3`, 4`-trimethoxyflavone, apigenin and its 7-*O*-β-D-glucopyranoside were isolated from the ethyl acetate extract. From the methanol extract, a new flavonoid, apigenin 7-*O*-(6``-*O*-*p*-hydroxybenzoyl)-β-D-glucopyranoside (1), along with vicenin 2, acacetin 7-*O*-rutinoside, and 6-hydroxyapigenin 7-*O*-β-D-glucopyranoside were isolated. Toxicity study of *n*-hexane, dichloromethane, ethyl acetate extract and methanolic extracts of the plant proved that it is relatively nontoxic. The tested extracts showed significant analgesic and anti-inflammatory effects as compared with control groups and reference drugs. Also, the tested extracts showed significant antioxidant activity as compared with reference.

Keywords: *Malabaila suaveolens,* coumarins, flavonoids, bioactivity.

INTRODUCTION

Malabaila suaveolens Coss. belongs to the family Umbelliferae (1). Chemical constituents found in the family include: volatile oils, coumarins, acetylenes and flavonoids, while terpenes, sesquiterpenes and alkaloids are rare (2). Major volatile oil constituents reported in the fruits of *Malabaila secacule* are: *p*-cymene and *a*-phellandrene (3), while, bergapten, isobergapten, imperatorin, pimpinellin, isopimpinellin, umbelliferone and sphondin are reported in *Malabaila dasycarpa* and *M. graveolens* fruits (4), in addition to marmesin and dihydroimperatorin (5). Nothing is repotrted on *Malabaila suaveolens*. In the present study, we report on chemical constituents and bioactivities of extracts of the fruits of *M. suaveolens*, aiming at the assessment of its potential medicinal uses.

MATERIALS AND METHODS

General

NMR spectra (JEOL EX 270 MHz NMR spectrometer), mass spectra (JEOL JMS-AX 500 was used for EI-MS, (±) ESI-MS Thermo (LCQ Advantage Max), GC/MS spectrometer: GC/MS finngigan Mat SSQ 7000, digital DEC 3000, GLC instrument used was Agilent 6890N gas chromatograph provided with FID (Flame Ionization Detector). EI ev 70, fused silica capillary column 30 m length, Helium gas as a carrier gas; flow-rate (column head pressure 13 PS) and MS detector and UV spectra (OMM 7070E Shimadzu UV 240 spectrophotometer) were run.

Plant material

Malabaila suaveolens Coss. fruits was purchased from Herbal Bazar at Cairo and identified by Dr. M. El-Gebali

(Department Phytochemistry and Plant Systematics, NRC, Cairo, Egypt).

Preparation and GC/MS analysis of volatile oils

Volatile oil was prepared by hydrodistillation of *M. suaveolens* fruits and analyzed by GC/MS system equipped with willey 138 and NBS 75 library software was used capillary GC using DB-5 column. Injection volume was 1.0 ul at 1:50 split. Ionization voltage 70ev scan mass range 40–400, with a temperature program 40°C/5min., 40–160, 3°C/min. 160–300, 5°C/min. The essential oils were identified by matching their spectra with those recorded in the MS library and comparison with those of reference compounds.

Preparation and GLC analysis of unsaponfiable matter (USM) and fatty acids (FA).

n-Hexane extract (7g) was saponified to yield the USM fraction (2.8 g) and FA fraction (2.1 g). The FA fraction was methylated by refluxing in 50 ml absolute methanol and 1.5 ml conc. sulphuric acid for 2hrs and analysed by GLC. The column used was a capillary column 30m length, 0.53 mm internal diameter, film thickness 0.50 μm, packed with 5% phenyl-95% dimelhylpolysiloxane. The analysis was carried out at a programmed temperature: intial temperature 80°C (Kept for 1 min), then increasing at a rate of 8°C/min. and final temp. 250°C (kept for 20 min.). Injector temp. was 280°C and detector temp. at 300°C, N_2 was as carrier gas at flow-rate 30ml/min; H_2 flow, 30 ml/min. and air flow-rate, 300 ml/min. For GLC of USM fraction, the column used was a capillary column 30m length HP-INWAX. Polyethylene glycol, 320 μm internal diameter, 0.25μm film thickness. The analysis was carried out at a programmed temperature: Intial temperature 70°C for 2 min. then increasing at a rate of 4°C/min. till 220°C, N_2 was used as carrier gas at flow-rate, 30 ml/min., and air flow-rate, 230 ml/min. for GLC of FA.

Extraction and isolation

The air-dried powdered *Malabaila suaveolens* fruits (2.5 kg) was extracted with *n*-hexane, dichloromethane, ethyl acetate and methanol, in succession to afford 105, 65, 33 and 120 g of extracts, respectively. The dichloromethane extract was subjected to silica gel column (100 × 4cm, 200g) and eluted with benzene and benzene / ethyl acetate step gradient, 50 ml fractions being collected were examined by TLC using solvent system benzene / ethyle acetate (8:2). As a result, fractions eluted with benzene afforded angelicin, fractions eluted by benzene/ethyl acetate (9.5 : 0.5) afforded 4,7,9-trimethylpsoralene. Finally the fractions eluted by benzene/ethyl acetate (9:1) afforded isopimpinellin and umbelliferone.The ethyl acetate extract was subjected to polyamide 6S column. The column was eluted with water / methanol step gradient. The obtained fractions were inspected by paper chromatography using BAW and 15% Ac.OH as developing systems. The similar fractions were collected together. Subsequent purification by Sephadex LH-20 CC afforded apigenin 7-*O*-*β*-D-glucopyranoside, 5-Hydroxy 7, 3`,4`-trimethoxyflavone and apigenin.

The methanolic extract (95 g) was subjected to polyamide 6S column. The column was eluted with water and water / methanol step gradient. The obtained fractions were subjected to PC using BAW and 15% $AcOH/H_2O$ as developing systems. The similar fractions were collected together to afford four compounds. Subsequent purification by sephadex LH-20 afforded vicenin 2, acacetin 7-*O*-rutinoside, 6- hydroxyapigenin 7-*O*-β-D-glucopyranoside, and a new compound, identified as apigenin 7-*O*-(6``-*p*-hydroxybenzoyl)-β-D-glucopyranoside (1).

Animals

Adult male albino rats weighting (120–150g) and adult albino mice of both sex (20–25 g) were used in this study, obtained from the animal – breeding unit of National Research Centre, Cairo, Egypt. The animals were fed a standard laboratory diet. The study was performed according to the international rules and to the guidelines of ethical comity of National Research Centre for experimental animal use.

Chemicals and references

Acetylsalicylic acid (El Nasr Co., Egypt), ibuprofin (Egyptian Int. pharmaceutical Industries Co., Tenth of Ramadan City, Egypt), Carrageenan (BDH, England). All other chemicals used in the experimental work were in analytical grade.

Pharmacological screening

The residues of the tested extracts were suspended in 7% Tween 80 and biologically tested in different doses. All doses were expressed in terms of extract weight/animal body weight (6). Acute toxicity (LD_{50}) of extracts was determined in mice as described previously (7). They were S.C. administered in doses ranging from 4 to 13 g/kg b. wt. Animals were observed and the mortality rates were recorded within the first 24 hrs after administration. Analgesic activity was evaluated in comparison with acetylsalicylic acid (as a central analgesic) (8). Anti-inflammatory activity was studied using carrageenan-induced rat's paw edema (9).

Groups of 18 hrs fasted male rats (110–130 g) of six animals each were orally dosed with either one of the terted extracts, 1h before carrageenan challenge foct paw edema was induced by subplanter infection of 0.05 ml of 1% suspension of carrageenan in saline into the planter tissue of one hind paw. An equal volume of saline was injected into the other hind paw and served as control. Four hours after extract, the animals were sacrificed and the hind paws were rapidly amputated at the tibiotarsal joint and weighed (8 & 10). The percentage of edema as well as of protection were calculated. Ibuprofen was employed as a reference against which the tested extracts were compared (11).

RESULTS AND DISCUSSION

GC/MS analysis of the essential oil obtained from *Malabaila suaveolens* fruits revealed the presence of 39 compounds (Table 1). It was found that hexyl butanoate (41.37 %), *n*-octyl acetate (15.92 %) and hexyl-2-methylbutanoate (7.57 %) were the major compounds. It was concluded that the volatile oil was rich in oxygenated hydrocarbons (98.37 %) and ester compounds (87.04 %). Saponfication of the *n*-hexane extract afforded the fatty acids (FA) fraction, as well as unsaponfiable matter (USM) fraction. GLC analysis of FA revealed the presence of 10 compounds. The total identified saturated fatty acids (76.71 %) was higher than that of unsaturated fatty acids (4.97 %); palmitic and pentadecanoic acids were the major saturated fatty acids (59.98 % and 8.99 %, respectively); Tetracosenoic and linoleic acids were the major unsaturated fatty acids (2.03 % and 1.97 %, respectively). GLC of USM fraction revealed the presence of 25 compounds. The total identified compounds representing (78.35 %) and involved cholesterol (0.75 %), β-sitosterol (2.97 %), campesterol (4.87 %), stigmasterol (14.94 %) and β- amyrin (0.80 %), in addition to long chain hydrocarbons. From dichloromethane extract four known coumarins were isolated and identified as angelicin (12), 4, 7, 9-trimethyl psoralen (13), isopimpinellin (14) and umbelliferone by comparing their UV, MS and NMR spectral data with the corresponding literature data. The flavonoids apigenin 7-O-*β*-D-glucopyranoside (15), 5-hydroxy 7,3`,4`-trimethoxyflavone and apigenin (16) were isolated and identified from the ethyl acetate extract. Chromatography of the methanol extract afforded a new flavonoid compound 1, in addition to vicenin 2 (17), acacetin 7-*O*-rutinoside (18), 6-hydroxyapigenin 7-O-*β*-D-glucopyranoside (19).

Compound 1 showed chromatographic properties and UV spectral data with shift reagents similar to those of a flavone with free hydroxyl groups at 4` and 5 positions and substitution at 7 position (16). Glucose and apigenin resulted by complete acid hydrolysis and identified by using Co-PC, compared with authentic samples. Positive ESI/MS spectrum of compound 1 showed $[M+H]^+$ at m/z 553 corresponding to $C_{28}H_{24}O_{12}$. The ^{1}H NMR spectrum of compound 1 showed a singlet signal at δ 12.97 proton of 5-OH group, two overlapping protons as a doublet signal at δ 7.95 with (J = 8.6 Hz) for H-2` and H-6`, two overlapping protons as a doublet signal at δ 7.51 with (J = 8.4 Hz) for H-2``` and H-6``` (*p*-hydroxybenzoate), two overlapping protons as a doublet signal at δ 6.89 with (J = 8.7 Hz) for H-3` and H-5`, a doublet signal at δ 6.81 for H-8 with (J = 2.5 Hz) and a doublet signal at δ 6.67 for H-6 with (J = 2.5 Hz). The ^{1}H NMR spectrum showed a singlet signal at δ 6.41 for H-3 and two overlapping protons as a

Table 1: GC/MS analysis of volatile oil constituents of *Malabaila suaveolens* fruits.

Identified Compounds*	R_t(min.)	Relative Area %
Hexanal	6.31	0.09
Ethyl isopropyl ether	7.15	0.14
1-Hexanol	9.80	1.52
Isopropyl 3-methyl butanoate	10.73	0.19
Butyl isobutyrate	13.74	0.58
n-Butyl n-butyrate	16.04	0.77
1-Octanal	16.39	0.57
1-Hexyl acetate	16.93	0.89
α- Phellandrene	17.23	0.49
Butyl 2-methyl butyrate	18.35	0.75
n-Butyl isovalerate	18.68	0.78
Limonene	18.91	0.51
1-Octanol	20.23	2.04
Hexyl propanoate	21.65	0.69
Hexyl 2-methyl propanoate	23.91	5.77
Hexyl butanoate	26.70	41.37
n-Octyl acetate	27.46	15.92
p-Allylanisole	28.01	4.04
Hexyl 2-methyl butanoate	28.37	7.57
Hexyl isovalerate	28.55	1.09
2-Cyclohexen-1-on,3-ethyl-6-(1-methylethyl)-	28.97	0.15
Thymol	30.77	0.12
Carvacrol	31.14	0.25
Allyl butyrate	32.31	0.25
Butanoic acid, 1-ethenylhexyl ester	32.97	1.80
n-Octyl isobutyrate	33.99	0.13
2-Butyl-2-octenal	34.26	0.58
Butyric acid, 1-pentylallyl ester	34.84	3.47
Hexyl hexoate	35.02	2.35
Octyl butyrate	35.18	0.09
3-Decen-1-ol	35.71	0.14
1-Dodecanol	36.15	0.32
Octyl-2-methyl butanoate	36.79	2.83
n-Octyl 2-methyl butyrate	36.96	0.32
4-Methyl-2-pentyl acetate	38.83	0.10
3-(2,2-Dimethyl propylidene) bicycle[3.3.1.] nona-2,4, dione	39.40	0.09
Benzylhydryl vinyl ether	41.82	0.08
1,3,-Diformyl-4,5-diacetyl cyclopentane	42.14	0.11
Hexanoic acid, 10-undecen-1-yl ester	42.60	0.42

* Identification was achieved by comparison of *Kovat index* (KI) with those obtained from the NIST/NBS libraries spectra and those reported by Adams (20)

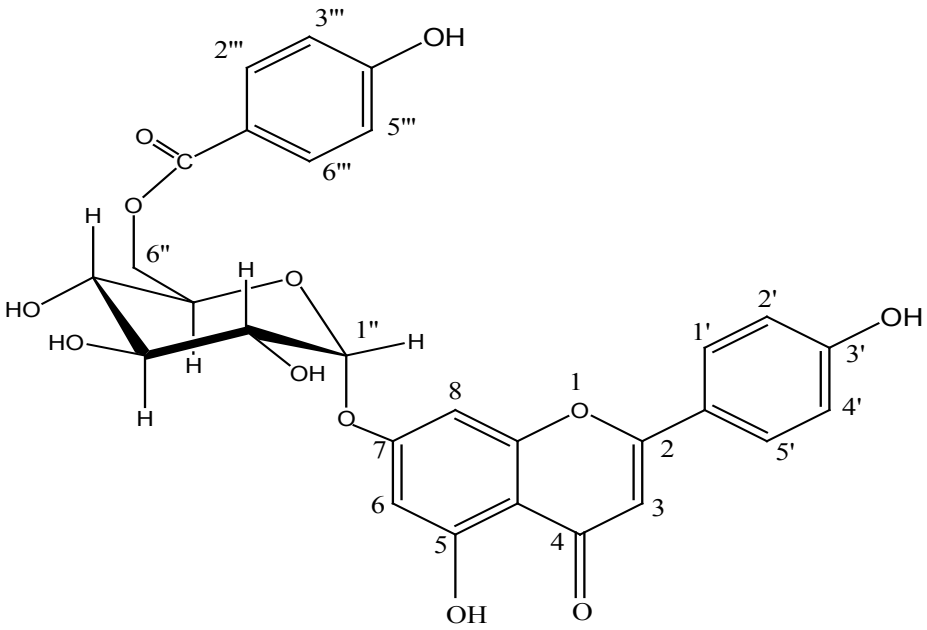

Figure 1: Structure of compound (1)
Apigenin 7-O-(6``-p-hydroxybenzoyl)-β-D-glucopyranoside (1)
Yellow powder, 12 mg, ESI-MS *m/z* 553 $[M+H]^+$; UV (MeOH) λ_{max}: 270, 320; +NaOMe 270, 310sh, 380; +NaOAc 272, 320, 395; NaOAc+H_3BO_3 270, 320 395sh; $AlCl_3$ 270, 320, 340, 355sh; $AlCl_3$+HC 270, 320, 350, 380sh; ^{1}HNMR (DMSO-d_6, 270 MHz) δ 12.97 (1H, s, 5-OH), δ 7.95 (2H, d, *J* = 8.6 Hz; H-2`,6`), 7.51 (2H, d, *J* = 8.4 Hz; H-2``` , 6```), 6.89 (2H, d, *J* = 8.7 Hz; H-3`,5`), 6.81 (1H, *J* = 2.5 Hz; H-8), 6.67 (1H, d, *J* = 2.5 Hz; H-6), 6.41 (1H, s, H-3) 6.29 (2H, d, *J* = 8.4 Hz; H3-```,5```), 5.17 (1H, d, *J* = 6.9 Hz; H-1``), 4.46 (1H, dd, *J* = 12.0, 3.0 Hz; H-6``a), 4.18 (1H, dd, *J* = 12.0, 5.0 Hz; H-6``b).

doublet signal at δ 6.29 with (*J* = 8.4 Hz) for H-3``` and H-5``` (*p*-hydroxybenzoate). The spectrum displayed also, the signal of anomeric proton of glucose as a doublet at δ 5.17 with (*J* = 6.9 Hz). The relatively down field shifts of a double doublet signal at δ 4.46 and triplet signal at δ 4.18 corresponding to H-6``a and H-6``b of glucose indicate that C-6`` of glucose was occupied by *p*-hydroxybenzoic acid. Thus, compound 1 was identified as apigenin 7-*O*-(6``-*O*-*p*-hydroxybenzoyl) *β*-D-glucopyranoside (Figure 1) and is reported here for the first time in nature.

Bioassay

Study of the acute toxicity of the tested extracts of *Malabaila suaveolens* fruits, assayed up to 4.o g/kg did not prove to be toxic, since they did not induce mortality or toxic main festafions in mice up to 24 hours post treatment. The LD_{50} of the tested extracts are presented (5.917, 5.083, 5.750 and 5.0 g/kg for *n*-hexane, dichloromethane, ethyl acetate and methanol, respectively.

The analgesic activity of the extracts are presented in (Table 2). It could be deduced that the ethyl acetate extract at a dose level of 600 mg/kg exhibited the highest analgesic activity as compared with the control value. In addition, the data revealed that, the dichloromethane extract (500 mg/kg) and the methanolic extract (650 mg/kg) exhibited similar analgesic activities. Also, the *n*-hexane extract at a level of (700 mg/kg) exhibited a significant activity compared with the control value. The analgesic activity of ethyl acetate extract can be possible be attributed to its flavonoid constituents. Results of the anti-inflammatory activity of extracts are compiled in (Table 3). It could be concluded that, the *n*-hexane extract (700 mg/kg) exhibited the highest anti-inflammatory activity compared with ibuprofin (35 mg/kg) used as reference drug, as it significantly reduced the rat's paw weight edema to 38.46 ± 2.54 compared to control value (65.08 ± 1.69), protection percentage being 41%. Moreover, the results revealed that, dichloromethane extract (at a dose level of 500 mg/kg) and ethyl acetate extract (600 mg/kg) exhibited similar anti-inflammatory activity. On the other hand, the methanolic extract (at a dose level of 650 mg/kg) showed a relatively high anti-inflammatory activity. Table (4) illustrated the scavenging activity of DPPH radical due to its reduction by different concentrations of volatile oil, *n*-hexane, dichloromethane, ethyl acetate and methanolic extracts. The extracts were shown to scavenge the stable DPPH radical over a concentration range volatile oil (0.5,

0.25, 0.125, 0.0625 and 0.0312 mg/ml), *n*-hexane extract (0.6, 0.3, 0.15, 0.075 and 0.375 mg/ml), dichloromethane extract (0.5, 0.25, 0.125, 0.0625 and 0.0312 mg/ml), ethyl acetate extract (0.55, 0.275, 0.137, 0.0687 and 0.0437 mg/ml) and methanolic extract (0.5, 0.25, 0.125, 0.0625 and 0.0312 mg/ml). Maximal scavenging was found at 0.5, 0.6, 0.5, 0.55 and 0.5 mg/ml for volatile oil, *n*-hexane, dichloromethane, ethyl acetate and methanolic extracts, respectively.

Antioxidant activity

The scavenging activity of the volatile oil, *n*-hexane, dichloromethane, ethyl acetate and methanol extracts on DPPH (1, 1-diphenyl-2-picrylhydrazyl radicals was measured according to the method of (21) with some modifications. An aliquot of 0.5 ml DPPH radical (Sigma) in DMSO was added to test tubes with 1ml of different concentrations of the extracts. DM50 was used instead of extract sample as control, and tert-butylated hydroxyl quinone (TBHQ) was used as standard. The reaction mixture was vortex mixed at room temperature and absorbance (Abs) was measured at 520 nm immediately after mixing.

The scavenging activity (SA%) on DPPH radicals was calculated by the equation:

$$SA\ \% = 1 - \frac{Abs.\ in\ the\ presonce\ of\ sample}{Abs.\ in\ the\ absence\ of\ sample} \times 100$$

Statistical analysis

Data were expressed as mean ± s.e. Statistical comparison between different groups was carried out using one way analysis of variance (ANOVA) followed by multiple comparison test (post hoc Dunnett's) (22).

Table 2: Effect of extracts of *Malabaila suaveolens* fruits on acetic acid induced -writhing in mice.

Animal groups	Dose (mg/kg)	N° of Writhing [(b)]	Protection %
Control	-	29.60 ± 1.45[a]	-
Acetyl salicylic acid	200	6.00 ± 1.18*	79.73
n-Hexane extract	600	21.50 ± 0.81*[a]	27.36
	700	12.56 ± 0.72*[a]	57.57
Methylene Chloride extract	400	16.67 ± 0.50*[a]	43.68
	500	10.68 ± 0.69*[a]	63.92
Ethyl acetate extract	500	12.83 ± 1.14*[a]	56.66
	600	8.83 ± 0.87*	70.17
Methanol extract	550	14.83 ± 1.08*[a]	49.89
	650	10.07 ± 0.59*[a]	65.98

* Significantly different from control value at $p < 0.05$

[a] Significantly different from acetyl salicylic acid (200 mg / kg) value at $p < 0.05$

b Each value represents the mean (number of writhing) ± s.e. of the number of animals in each group (n = 6).

"Table 3: Anti-inflammatory activity of extracts of *Malabaila suaveolens* fruits using carrageenan -induced rat's paw edema.

Animal Groups	Dose (mg/kg)	% Increase in weight of paw edema (g) [b]	Protection %
Control	-	65.08 ± 1.69[a]	-
Ibuprofin	35	31.09 ± 1.84*	52.23
n-Hexane extract	600	48.28 ± 1.78*[a]	25.81
	700	38.46 ± 2.54*[a]	40.90
dichloromethane extract	400	56.42± 2.45*[a]	13.31
	500	50.64 ± 1.65*[a]	22.19
Ethyl acetate extract	500	58.29 ± 2.43*[a]	10.43
	600	49.92± 1.88*[a]	23.29
Methanol Extract	550	51.93 ± 2.41*[a]	20.97
	650	43.16 ± 1.11*[a]	33.68

* Significantly different from control value at $p < 0.05$.

[a] Significantly different from ibuprofin (35 mg / kg) value at $p < 0.05$.

[b] Each value represents the mean (% increase in weight of paw edema ± s.e of thenumber of animals in each group (n = 6).

Table 4: Antioxidant activity of extracts of *Malabaila suaveolens* fruits using DPPH radical.

Extract Concentration (mg/ ml)	Activity %
1-Volatile oil	
0.50	72.81 ± 6.92
0.25	45.65 ± 4.60
0.125	28.25 ± 1.93
0.0625	15.21 ± 2.11
0.0312	5.24 ± 0.54
2- *n*-Hexane	
0.60	28.14 ± 2.10
0.30	14.40 ± 0.65
0.15	6.95 ± 0.15
0.075	–
0.0375	–
3- Methylene chloride	
0.50	68.40 ± 1.54
0.25	49.41 ± 3.20
0.125	27.36 ± 2.60
0.0625	17.26 ± 2.12
0.0312	8.29 ± 0.19
4- Ethyl acetate	
0.55	70.90 ± 6.44
0.275	36.35 ± 1.56
0.137	17.25 ± 2.51
0.0687	7.12 ± 0.54
0.0312	3.12 ± 0.31
5- Methanol	
0.5	80.36 ± 2.39
0.25	50.54 ± 4.25
0.125	30.56 ± 1.11
0.0625	16.83 ± 0.88
0.0312	9.05 ± 0.51
Standard TBHQ	95.11 ± 1. 63

Values are means ±st. of triplicate measurements

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