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MACFADYENA UNGUIS-CATI (L.) A. GENTRY, A SOURCE OF FREE RADICAL SCAVENGER COUMESTROL

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ABSTRACT

Testing the successive extracts and coumarin- containing fraction isolated from *Macfadyena unguis-cati* for antioxidant activity by inhibiting the stable DPPH free radical, it showed high activity (93.9% inhibition) when compared with vitamin C (95.4% inhibition). Also successive extracts and 80% total ethanol extract showed moderate antimicrobial activity by inhibiting the growth of *Bacillus cereus* (gram-positive bacteria) and *E-coli* (gram-negative bacteria). Fractionation of coumarin containing-fraction led to isolation of two compounds, the first is the coumestrol, 7-hydroxy, 4`, 5` dimethoxy coumestan and the second is 6-methoxy-2-(4`-methoxy-2`-hydroxyphenyl)-benzofuran-3-carbaldehyde.

Keywords. *Macfadyena unguis-cati*, bignoniaceae, antioxidant activity, antibacterial activity.

INTRODUCTION

Macfadyena unguis-cati (L.) A. Gentry { Syn: *Doxantha unguis-cati*} (*Bignoniaceae)* is an ornamental climbing plant, widespread in Egypt, America and Western India. The plant is known in Arabic as Makhlab Al'kott, cat's claw. Cahoon *et al*. (1998), found about 80% palmitoleic acid (C_{16}) plus *cis* – vaccenic acid (C_{18}) in its seed oil. Root extracts of *M.unguis-cati* were found to contain lapachol, quinovic acid, 3-(O-fucosyl) alcohol, β-amyrin and β -sitosterol (Joshi *et al.*, 1985). Traces of cyanidin -3-glucoside were reported in flower extracts, (Scogin, 1980). On the other hand, leaf extracts of *M.cynanchoide*s contain the iridoids cynanchoside (Bonini *et al.*, 1981), Macfadienoside (Bianco *et al.*, 1974) and 5, 7-bisdeoxycynanchoside (Adriani *et al.*, 1982). Few reports have been published about iridoids in *Bignonieae;* being mainly C-4 carboxylated, Poser *et al*. (2000), while decarboxylated iridoids were reported in *Macfadyena cynanchoides*. Subramanian *et al*. (1972), examined the flavonoids of eight Bignoniaceous plants comprising *Bignonia gracilis* and *B.megapotamica Spreng*. They found quercetin-3-rutinoside in both, and quercetin-3-galactoside in the latter. The nectary structure and chemical nectar composition of 15 species of *Bignoniaceae*(*M.dentata,M.unguis–cati*,*Tecoma garrucha* and *T. stans*) were analyzed by Graletto (1995). Two aglycone moieties of the isocoumarin glycosides were isolated from the bark of *Tabebuia impetiginosa*, four iridoid glycosides, two lignan glycosides, three phenyl ethanoid glycosides, and eight phenolic glycosides (Warashina *et al.*, 2004). *M.unguis-cati* is used in folk medicine to treat snakebite (Houghton and Osibogun 1993), dysentery, inflammation and rheumatism (Pio Correa, 1978). In addition, there are reports on its use in the treatment of venereal disease and as a quinine substitute for malaria (Ferrari *et al.*, 1981). The extracts of the whole plant did not show antiprotozoal activity against *Leshmania* spp. or *Trypanosoma cruzi* (Fournet *et al.*, 1994). The biological screening of fractions derived from leaves and liana of *M.unguis-cati* revealed antitumor and antitrypanosomal activities. In addition, the anti-lipoxygenase and anti-cyclooxygenase observed in these fractions showed partial correlation with the anti-inflammatory property attributed to this plant (Duarte *et al.*, 2000).

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MATERIALS AND METHODS

Plant material: The fresh unflowering aerial part of *Macfadyena unguis-cati* F. *Bignoniaceae* was collected from Manial Palace, Manial, Cairo in August 2005 and identified by Dr. Mohamed El-Gebaly (Plant Taxonomist).

Preparation of successive extracts: 500g of powdered air-dried unflowering aerial part of *M.unguis-cati* was extracted in a Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and ethanol 95%, in succession. Another part of the dried powder was extracted with ethanol 80%. These extracts were evaporated to dryness under vacuum at 40°C, yielding dark oily residues.

Preparation of coumarin-containing fraction: The dried powdered aerial parts of *M.unguis-cati* (500g) was exhaustively extracted by percolation with 80% ethanol. The concentrated extract was treated with an equal volume of 10% KOH solution at room temperature for 1 hr. The alkaline alcohol extract was diluted with water and extracted with ether. The aqueous layer was acidified with dilute HCl, refluxed for 1.5 hrs. cooled and extracted with ether, whereby the ethereal extract was evaporated to dryness (coumarin fraction). This fraction was dissolved in ethanol and subjected to TLC using silica gel G60, F254 precoated plates, developed with benzene: ethyl acetate (8:2) and sprayed with I_2/KI reagent. Two blue spots were detected at R_f 0.51 (compound I) and 0.6

Table 1. Inhibition of DPPH radical by *M.unguis-cati* extracts:

Compound	Double integration area	Percentage inhibition
DPPH	639.4	-
Vitamin C	29.27	95.40
Petroleum ether ext.	564.4	11.70
Chloroform ext.	511.6	19.98
Ethanol ext.	320.4	49.89
Coumarin fraction	42.7	93.90

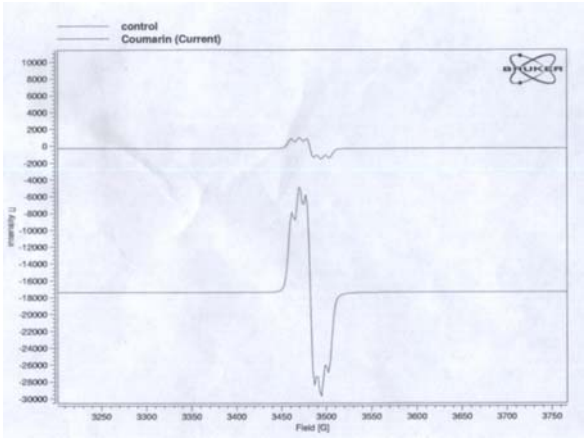


Fig 1. ESR. of control DPPH and coumarin fraction of *M.unguis-cati*.

(compound II), the color being intensified by 1_2/KI, purified by preparative layer chromatography using the same system and recovered from silica gel by chloroform, evaporated under reduced pressure at 40°C and them subjected for spectroscopic analysis, (MS, IR, UV, ^{1}HNMR and ^{13}CNMR).

Apparatus:

- Mass spectrometer Finnigan Mat SS Q 7000, Digital DEC EL, 70 eV.
- ^{1}HNMR, Spectrophotometer Jeol EX-300 NMR spectrometer.
- ^{13}CNMR Spectrophotometer Jeol EX-300, 75 MHz.
- Ultra violet absorption spectrophotometer (Shimatzo).
- ESR Bruker (Germany).
- IR Bruker Victor- -22

Characterization of compounds:

Two compounds were isolated from the coumarin-fraction of the aerial part of *M.unguis-cati*. Both showed blue color under UV, intensified by spraying with I/KI reagent.

Compound I:
It was obtained as orange powder, R_f 0.51 (benzene: ethyl acetate, 8:2), UV $\lambda_{max}^{methanol}$: 230, 268, 311, 330; methanol/KOH: 236, 268, 348, 388 IR cm^{-1} (K Br. disc): 3445 br, 2932, 1848, 1797, 1700, 1633, 1525, 1458, 1403, 1250, 1126, 1065, 1013, 920, 619, 421. EIMS: ($^m/_z$, rel. int.), $C_{17}H_{12}O_5$, 312 M^+ (7%), 301(7%), 288 (10%), 262 (11%), 259(17%), 233 (38%), 213 (17%), 203 (15%), 177 (25%), 149 (24%), 135 (27%), 133 (47%), 122 (58%), 105 (100%), 91(50%), 73 (74%), 60 (92%).

^{1}HNMR: (300 MHz, $CDCl_3$), δ ppm 3.938, 3.978 (6H,-OCH_3, 4`,5`); 6.513-6.551-(1H,d.d. $J_{6,5}$ =9.0,$J_{6.8}$ =2.1, H-6); 6.838-6.831 (1H,d.$J_{8,6}$=2.1, H-8); 7.048 (1H,s, H-3`); 7.601 (1H,s,H-6`); 7.825-7.795 (1H,d.$J_{5,6}$=9.0, H-5).

^{13}CNMR: (75 MHz, $CDCl_3$) 56.4 (OCH_3), 77.3 (C-3), 82.0 (C-4), 90.6 (C-3`),100.0(C-8), 105.2 (C-6), 116.1(C-10), 128.3(C-1`), 129.1 (C-6`), 130.9 (C-5), 152.2 (C-9), 149.2 (C-7), 159.9 (C-2`), 163.8 (C-5`), 161.8 (C-4`), 168.7 (C-2).

Compound II:
It was isolated at R_f 0.6 (benzene: ethyl acetate, 8:2) as white powder, with blue color which turned to dark blue by using 1/kI spray reagent.

UV $\lambda_{max}^{methanol}$:274, 330; methanol/KOH: 277, 302 sh, 380.
EIMS (70eV), ($^m/_z$ rel. int.): $C_{17}H_{14}O_5$, 298 (M^+, 5%), 283(10%), 279 (17%), 262(19%), 260(14%), 233 (38%), 203 (19%), 177 (21%), 167 (42%), 164 (30%), 149 (100%), 142 (25%), 133 (42%), 122 (20%), 105 (26%), 97 (24%), 89 (53%), 87 (70%), 82 (32%), 73 (48%), 60 (97%), 57 (65%), 55 (55%).

Investigation of antimicrobial and antioxidant activities:

Antimicrobial screening of successive extracts of *M. unguis-cati* :Successive extracts of the dried aerial part of the plant, were prepared using petroleum ether, ether, chloroform, ethyl acetate and ethanol (yield, 2, 2.83. 0.916, 1.96 and 3.33 % w/w respectively of solvent-free extracts). In addition, the powder was macerated in 80% ethanol to produce total ethanol extract (10.5 %). Evaporated extracts were tested for their

antimicrobial activity against certain bacteria and yeast. Tests were carried out using the diffusion assay method Hammond and Lambert (1978); Hashem and Saleh (1999); Hashem and Wahba (2000).

The diameter of the cups made was 0.8 cm and concentrations used were 10 mg/1 mL, (0.5g %) ampicillin (Wyeth) and 0.5g% Clotrimazole (Bayer), 10 mg of each extract, suspended in 1 mL of 10% Tween 80 as diluent vehicle. The capacity of the cup was 0.02 mL. Dishes seeded with test organisms, incubated at 37^{o} (bacteria) and at 30^{o} (yeast). Diameters of inhibition zones were measured in mm, after incubation for 24-28 hrs.

Microbial strains: Pure strains of microbes were kindly obtained from the Microbial Genetics Department, NRC, Dokki, and Cairo.

Evaluation of antioxidant activity using DPPH as stable free radical:
(Makhmoor, 2003 and Aqua and Innocenti, 2004).

10 mg of each successive extract, total ethanol extract and coumarin containing-fraction was dissolved in 1 mL methanol. DPPH was prepared in concentration of 10 mg/mL methanol (as source of stable free radical). Standard solution of vitamin C as antioxidant (positive control), (10 mg/ mL methanol). Test solution (10 µL) and standard (10 µL) were added to 190 µL of DPPH, the control was adjusted to 0.2 mL DPPH. They were all incubated at 37^{o}C for 30 min. Electron Spin Resonance of DPPH was recorded, and the percentage inhibition of the free radical was calculated from the double integration areas.

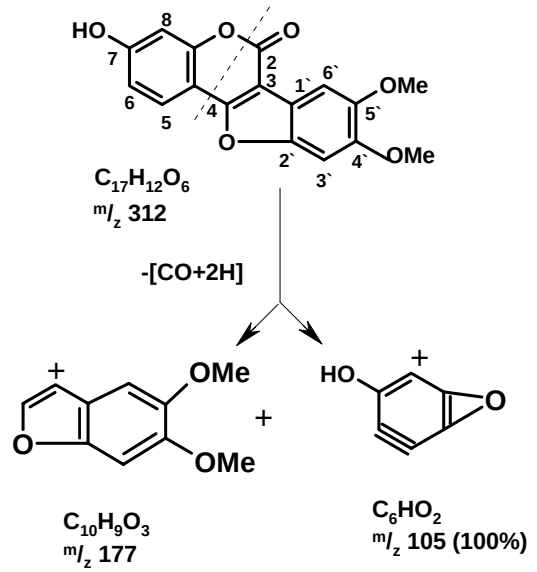
RESULTS AND DISCUSSION

Two compounds were isolated from the coumarin fraction of the aerial part of *M.unguis-cati*, appearing blue on silica gel plates which intensified with I/KI spray reagent.

Compound I: was obtained as orange powder, mp. 215-217^{o}C. The UV absorption bands at 268 and 311 have been attributed to the benzene and pyrone rings, respectively. Absence of bathochomic shift of the maximum at 268 nm, after addition of KOH indicates no methyl substitution at C-5, C-7 or C-8. Strong absorption at 230 nm indicates 7-hydroxy coumarin. The bathochromic shift of the band at 330 to 388 (*ac* 58 nm), after addition of alkali, is attributable to 7-hydroxy group. Infrared spectroscopy of this compound showed broad band at 3445 cm^{-1} due to 7-hydroxy and 1700 cm^{-1} for C=O stretching. Strong absorption band at 1633 cm^{-1} is attributed to C=C skeletal vibration which starts from 1613 to 1639 (for C=C stretching of furan ring). Band at 1250 cm^{-1} in the region of (1237-1272) is ascribable to methoxy compounds. Bands at 1065 and 1250 cm^{-1} could be ascribed to C-O stretching frequencies of furan ring.

Mass spectroscopic analysis indicated the molecular weight ($^{m}/_{z}$ =312) and the molecular formula $C_{17}H_{12}O_6$.

The base peak at m/z 105 and presence of peak at m/z 177 indicated the following fragmentation pattern from which the structure was suggested to be 7-hydroxy, 4`, 5` dimethoxy coumestan



The structure was confirmed by ^{1}HNMR spectroscopy.

^{1}HNMR spectrum exhibited signals corresponding to two methoxy substituents in an aromatic system at δ ppm 3.938 and 3.978. Signals corresponding to the A ring are those at δ ppm 6.513-6.551 (d.d.) with $J_{6,5}$ = 9.0 and $J_{6,8}$ = 2.1 for H-6. The doublet signal at 6.838-6.831 with $J_{8.6}$ =2.1 is due to meta coupling of H-8.

The doublet signal at δ ppm 7.825-7.795 with J= 9.0 (*ortho* coupling) for H-5, H-3` and H-6` protons appeared at δ ppm 7.048 and 7.601.

Compound II: was isolated in very small amount as amorphous powder. The mass spectroscopic analysis of this compound was found in agreement with the data published by Macias, *et al*., 1999 for melimessanol C which has the structure 6-methoxy-2-(4`-methoxy-2`-hydroxyphenyl)-benzofuran-3-carbaldehyde, molecular wt. 298 (M^{+}, $C_{17}H_{14}O_5$) with the characteristic fragments at 283, 279 and 149 (100%).The biogenesis of the compound should come through the C ring opening in a coumestan precursor.

The antioxidant activities of successive extracts of *M.unguis-cati*, (petroleum ether, chloroform, ethanol and

the coumarin containing fraction) were estimated by inhibiting the stable DPPH free radical. The results obtained from recording the double integration areas of DPPH by ESR were calculated after the addition of the inhibitor (extract). The most potent extract is the coumarin containing-fraction with 93.9% inhibition when compared with vitamin C (95.4%)[Fig. 1 and Table 1].

The most common natural antioxidants are phenolic acids and flavonoids (Larson, 1988), these are not only the defensive molecules in prevention of different pathological disorders but are commonly used in industry for the prevention of oxidative degradation of polymers and synthetic and natural pigments. Radicals have been proposed to induce cellular damage which may play a role in heart diseases rheumatoid arthritis, cancer, and inflammatory disorders as well as in aging processes (Heilmann *et al*., 1995).

Antimicrobial screening of successive extracts and 80% total ethanol extract of *M.unguis-cati* revealed moderate activity against *Bacillus cereus* (gram-positive bacteria) and also showed mild inhibition of the growth of *E.coli* (gram-negative bacteria), while not affecting the growth of the yeast *Candida albicans.*

These results produced from the preliminary antimicrobial screening test proved that, this plant is of very low toxicity and can be used in large doses and for long time as anti-inflammatory and antioxidant. These results are confirmed also from the results of LD_{50} of 80% total ethanol extract of *M.unguis-cati* which was found to be 4.9 g/kg b. wt. and published in a previous work (Aboutabl *et al*., 2006).

The plant is of significant potential as antioxidant, anti-inflammatory agent, taking in consideration the relative safety of its 80% ethanol extract (LD 50=4.9g/kg b wt). Aboutabl *et al.* (2006)

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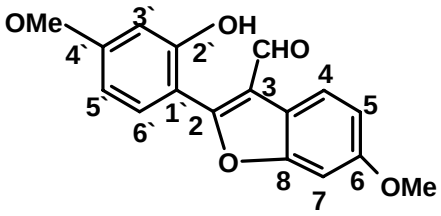

6-methoxy-2-(4`-methoxy-2`- hydroxyphenyl)-benzofuran-3-carbaldehyde

$C_{17}H_{14}O_5$(298)

Table 2. Antimicrobial activity of 80% ethanol and successive extracts of *M.unguis-cati*.

Organism	Diameter of inhibition zone (cm).						
	80% total ethanol ext.	Pet. Ether ext.	Chlorofo rm ext.	Ethyl acetate ext.	Ethanol ext.	Ampici-llim	Clotrima-zole
• Gram-negative bacteria:							
1- *Escherichia coli*	2.2	1.8	1.7	1.4	1.8	6.5	-
2- *Pseudomonas aeruginosa*	-ve	-	-	-	-	4.5	-
• Gram-positive bacteria:							
1- *Bacillus subtilis*	-	-	-	-	-	5.0	-
2- *Bacillus cereus*	2.0	2.3	2.4	1.4	1.4	3.25	-
3- *Staphylo-coccus aureus*	-	-	-	-	-	5.0	-
• Yeast:							
Candida albicans.	-	-	-	-	-	-	5.0
LSD 5%	0.096	0.155	0.490	0.329	0.124	1.098	
LSD 1%	0.222	0.971	1.135	0.758	0.287	1.597	

Mean of three tests.
LSD is the least significant difference according to "t" Student test. (Snedecor and Cochran 1971)

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