INT. J. BIOL. BIOTECH., 3 (4): 695-702, 2006.

PHYTOCHEMICAL AND BIOACTIVITY INVESTIGATIONS OF *MACFADYENA UNGUIS-CATI* L. (BIGNONIACEAE)

E.A. Aboutabl[1], F.A. Hashem[2], A.A. Sleem[3] and A.A. Maamoun[2]

[1]*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street, Cairo, Egypt.*
[2]*Pharmacognosy Department, National Research Centre, Tahrir Street, Dokki, Cairo, Egypt.*
[3]*Pharmacology Department, National Research Centre, Tahrir Street, Dokki, Cairo, Egypt.*

ABSTRACT

GC/MS of the volatile components of the aerial part of *Macfadyena unguis-cati* L, Fam.. Bignoniaceae revealed 74 compounds, 52 of them (representing 75.97%) were identified. The major compound is n-decane (12.21%) followed by phytol (12.19%). The saponifiable fraction of the petroleum ether extract showed 21 fatty acid identified as methyl esters. 37 compounds were identified in the unsaponifiable fraction;representing 93.26%. β-amyrin, squalene, β-sitosterol and 3ά,5-cyclo-ergosta-7,22-dien-6-one were identified in the USM.Determination of LD_{50} of different extracts showed that total ethanol extract is the safest (4.9 g/kg) followed by petroleum ether extract, (4.5 g/kg) and ethyl acetate extract having the least LD_{50} (3.1 g/kg). The total ethanol extract was revealed to be the most potent as antipyretic, followed by ethyl acetate extract. The ethanol extract, as well as the coumarin containing fraction exhibited significant analgesic activity.

Key-words: *Macfadyena unguis-cati* L., Bignoniaceae, biological activity, fatty acids, unsafonifiabale matter.

INTRODUCTION

Macfadyena unguis-cati L. A. Gentry or *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 *Bignoniaceae*, 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 analysed by Graletto (1995).

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 activities observed in these fractions showed partial correlation with the anti-inflammatory property attributed to this plant (Duarte *et al*., 2000).

MATERIALS AND MATHODS

Plant material

Fresh leaves of *Macfadyena unguis-cati L.* Fam. Bignoniaceae was collected from Manial Palace, Manial, Cairo in August 2004 and identified by plant taxonomist Dr. Mohamed El-Gebaly.

Preparation of volatiles

Fresh leaves were chopped and subjected to combined hydrodistillation/solvent extraction using a modified Likens and Nickerson apparatus with 2-methyl butane (15mL) as a solvent.

Preparation of successive extracts

500 g of powdered air-dried leaves 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 95%. These extracts were evaporated to dryness under vacuum at 40^{o}C yielding dark oily residues.

Investigation of lipid content

The residue obtained after evaporation of petroleum ether was subjected to saponification by 0.5 N alcoholic KOH (500mL) and refluxing for six hours, cooled and concentrated under vacuum, then mixed with 100 mL water. The unsaponifiable matter was extracted exhaustively with ether. The combined ethereal extracts was washed with water till free from alkalinity, dried over anhydrous sodium sulphate, evaporated to dryness, and subjected to GC/MS analysis. The saponifiable part containing fatty acids, was dissolved in 50 mL of absolute methanol containing 5% HCl and refluxed for three hours, cooled, diluted with water and extracted with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness, then subjected to GC/MS for fatty acid methyl esters.

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 one hour. 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 1_2/KI reagent. One blue spot was detected at R_f = 0.51, the colour being intensified by 1_2/KI.

GC/MS analysis

A Hewlett-Packard 5840 gas chromatograph directly coupled to a mass spectrophotometer, Finnigan with FID was used. For the analysis of volatile compounds, a capillary column of DB-5 fused silica, 30 m x 0.25 mm id. and 0.25 μ m thickness; carrier gas: Helium at 30mL/min; temp. programming: 40^{o}-250^{o} at a rate of 5^{o}/min; ion source temperature, 180^{o}; ionization voltage 70 eV was used. DB-17 Column was used for GC/MS of fatty acids and DB-5 for unsaponifiable matter (sterols and hydrocarbons) with temp. prog. 60-280 ^{o} at a rate of 3 ^{o}/min.

Investigation of biological activities

Experimental animals

Adult male albino rats weighing 130-150 g and albino mice of 25-30 g body weight, were obtained from the animal house of National Research Centre, Egypt. They were kept under hygienic conditions and well- balanced diet and water.

Normal diet: vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95%pure) 10.5% and starch 54.3%.

Drugs: Paracetamol as positive control for antipyretic activity and dipyrone metamizol as positive control for analgesic activity. Doses of the drugs used were calculated according to Paget and Bernes, (1964) and were administered orally by gastric tube.

Determination of LD_{50}: It was carried out by ethanol extract of *M.unguis-cati* following Miller and Tainter 1944, procedure. Albino mice (25-30 g) were divided into groups, each of 6 animals. Preliminary experiments were carried to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses; each dose was injected in a group of 6 animals. The number of dead animals in each group, 24 hrs. after injection was recorded and LD_{50} was calculated.

Antipyretic activity: was carried out following the method of Bush and Alexander, 1960. Thirty six female albino rats of average body weight (130-150 g) were used, being subdivided into six groups (six animals each). The normal vaginal temperature was recorded before starting the experiment. Pyrexia was induced by intramuscular injection of 1 mL/100g b.wt. of 44% yeast suspension. The site of injection was then massaged to spread the suspension beneath

the skin. After 18 hours, the vaginal temperature was recorded for all groups to serve as the baseline of elevated body temperature, with which the antipyretic effect will be compared. One single oral administration of the tested extracts or paracetamol (positive control) was given in doses of 100 mg/kg b.wt., while the negative control groups received 1 mL saline. One and two hours later, vaginal temperature was recorded .

Analgesic activity: of the total ethanol extract and successive extracts of aerial parts of *M.unguis-cati*. was evaluated according to the method of Charlier *et al.*, (1961). Seven groups, each of six animals (adult male albino rats), were orally treated with the extract (100 mg/kg), dipyrone metamizol (positive control) and saline (negative control). An electric current as a noxious stimulus was used, where electrical stimulation was applied to the rats tail by means of 515 Master Shocker (Lafayette Inst. Co) using alternative current of 50 cycles / sec. for 0.2 second. The minimum voltage required for the animal to emit a cry was recorded for each group after one and two hours intervals of oral administration of the tested extracts.

Statistical analysis: The obtained data was statistically analysed using student's "t" test (Snedecor and Cochran, 1971).

RESULTS AND DISCUSSION

Composition of volatiles

GC/MS of the volatile components of *M.unguis-cati* aerial parts showed 74 compounds (Table 1), 52 of them representing 75.97% of the total composition of the oil were identified (Adams, 1995). 17 compounds representing 23.35% are unoxygenated, while 35 compounds being oxygenated (52.62%). The major compounds are n-decane (12.21%), followed by phytol (12.19%), 3-hexanal (6.50%), pinan-2-ol <*cis*> (5.35%) and 9, 12, 15-octadecatrienoic acid ethyl ester (3.32%). It was found that the ketone compounds: damascenone <(E)-β>, damascone <(E)-α>, ionone <(E)- α> and ionone <E- β> are structurally related with M^+: 190, 192, 192 & 192, respectively, and base peaks: 69, 123, 121 & 43 respectively (Table 1). Damascone could be confirmed from its fragmentation pattern:

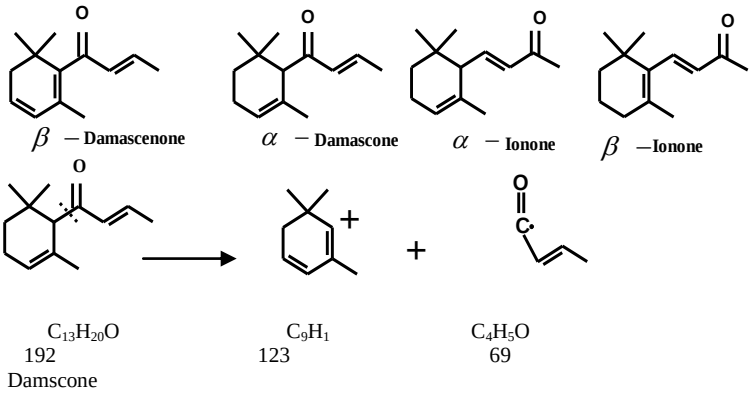


Table 1. Chemical composition of volatiles from aerial parts of *Macfadyena unguis-cati* L.

No.	Compound	Mol.formula.	Mol.wt.	Bp.	RRt	%
1	Heptane, 2- methyl	C_8H_{18}	114	43	0.25	2.80
2	Octene <1>	C_8H_{16}	112	43	0.28	0.42
3	Octane	C_8H_{18}	114	43	0.30	1.48
4	Butyl acetate <1>	$C_6H_{12}O$	116	43	0.32	1.04
5	3- Hexanal	$C_6H_{10}O$	98	41	0.46	6.50
6	*Cis*-3-Hexen-1-ol	$C_6H_{12}O$	100	41	0.50	1.50
7	n-Nonane	C_9H_{20}	128	43	0.54	2.41
8	3- Heptanone-5-methyl	$C_8H_{16}O$	128	43	0.86	2.06
9	Hexanal,2-2-dimethyl	$C_8H_{16}O$	128	57	0.95	0.82
10	n-Decane	$C_{10}H_{22}$	142	57	1.00	12.21

Table 1. Continued …….

No.	Compound	Mol.formula.	Mol.wt.	Bp.	RRt	%
11	Cineole	$C_{10}H_{18}O$	154	43	1.02	0.22
12	Ocimene <(Z)- β >	$C_{10}H_{16}$	136	93	1.07	0.36
13	Benzene acetaldehyde	C_8H_8O	120	91	1.11	0.33
14	Linalool oxide (*cis)*	$C_{10}H_{18}O_2$	170	59	1.19	1.29
15	3- Hexene-1-ol, propanoate	$C_9H_{16}O_2$	156	57	1.27	0.23
16	n-Nonanal	$C_9H_{18}O$	142	41	1.30	1.15
17	Pinan-2-ol <*cis*>	$C_{10}H_{18}O$	154	43	1.35	5.35
18	Pinene oxide < β >	$C_{10}H_{16}O$	152	67	1.59	0.81
19	Methyl salicylate	$C_8H_8O_3$	152	120	1.66	2.35
20	Anethole (Z)	$C_{10}H_{12}O$	148	148	1.67	0.60
21	Terpineol	$C_{10}H_{18}O$	154	59	1.68	1.33
22	3-Cyclohexene-1-acetaldehyde, α,4-dimethyl	$C_{10}H_{16}O$	152	94	1.72	0.70
23	*cis*-3-hexenyl 3-methylbutanoate	$C_{11}H_{20}O_2$	184	82	1.75	0.40
24	Ethyl salicylate	$C_9H_{10}O_3$	166	120	1.92	1.62
25	n-Tridecane	$C_{13}H_{28}$	184	57	1.99	0.24
26	Naphthalene,1,2-dihydro-1,1,6-trimethyl	$C_{13}H_{16}$	172	157	2.19	0.22
27	Damascenone <E- β >	$C_{13}H_{18}O$	190	69	2.29	0.76
28	Damascone <E- α >	$C_{13}H_{20}O$	192	123	2.30	0.63
29	Ionone <E- α >	$C_{13}H_{20}O$	192	121	2.43	0.29
30	Geranyl acetone	$C_{13}H_{22}O$	194	43	2.52	0.26
31	Ionone < β -E>	$C_{13}H_{20}O$	192	43	2.62	0.65
32	Pentadecane	$C_{15}H_{23}$	212	43	2.67	0.22
33	Nerolidol <E>	$C_{15}H_{26}O$	222	69	2.93	0.51
34	Hexenyl benzoate <(Z)-3->	$C_{13}H_{16}O_2$	204	105	2.94	0.53
35	Ethyl dodecanoate	$C_{14}H_{28}O_2$	228	88	2.98	0.30
36	n-Hexadecane	$C_{16}H_{34}$	226	43	3.27	0.25
37	n-Heptadecane	$C_{17}H_{36}$	240	57	3.29	0.38
38	Heptadecane,2-methyl	$C_{18}H_{38}$	254	43	3.40	0.22
39	5-Octadecene,(E)	$C_{18}H_{36}$	252	55	3.50	0.27
40	Ethyl tetradecanoate	$C_{16}H_{32}O_2$	256	88	3.57	0.37
41	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268	43	3.68	1.35
42	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	81	3.74	0.70
43	n- Nonadecane	$C_{19}H_{40}$	268	57	3.85	0.27
44	Isophytol	$C_{20}H_{40}O$	296	71	4.0	0.30
45	Dibutylphthalate	$C_{16}H_{22}O_4$	278	149	4.02	0.11
46	Phytol	$C_{20}H_{40}O$	296	71	4.44	12.9
47	9,12,15-Octadecatrienoic acid, ethyl ester,(Z,Z,Z)	$C_{20}H_{34}O_2$	306	77	4.52	3.32
48	Heptadecanoic acid, 15-methyl-, ethyl ester	$C_{20}H_{40}O_2$	312	88	4.55	0.75
49	Tetracosane	$C_{24}H_{50}$	338	57	4.79	0.79
50	Pentacosane	$C_{25}H_{52}$	352	43	5.03	0.56
51	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	149	5.10	1.30
52	Hexacosane	$C_{26}H_{54}$	366	57	5.21	0.27

RRt=Retention time relative to n-Decane=1.0

		II- Oxygenated compounds:	
Total unoxygenated compounds	23.35%	A. Alcohols	2.20
Aromatic compounds	6.46	B. Long chain ketones	3.41
Total oxygenated compounds	52.62	C. Long chain esters	6.41
Total identified compounds	75.97%	D. Long chain aldehydes	8.47
I-Unoxygenated compounds:		E- Monoterpene alcohols	7.97
A. Long chain hydrocarbons.	19.08	F. Monoterpene oxides	1.03
B. Branched hydrocarbons.	3.03	G. Monoterpene ethers	0.6
C. Unsaturated hydrocarbons**.**	0.69	H. Sesquiterpene alcohols	0.51
D. Monoterpene hydrocarbons	0.36	I. Other terpenoids	15.09

The configuration of α-damascone and α -ionone, important flavour components in black tea, was assessed by enantioselective capillary gas chromatography (König *et al*, 1989). In addition, the presence of 3-hydroxy- β-damascone was proved in several *Nicotiana* species (Kodama *et al*, 1984), while 4-hydroxy- β-damascone was

identified in the steam distillable oil from *Virginia tobacco* (Bolt *et al*, 1983). Moreover, Werkhoff *et al* (1991), described the enantiomeric resolution of *trans*-α-damascone and *trans*-α- ionone by inclusion gas chromatography of flowers of *Boronia megastigma* Nees and *Viola odorata* L. Williams *et al.* (1982) discovered the presence of damascenone and 1,1,6- trimethyl-1,2- dihydronaphthalene and their precursors in grape juice. Kotseridis *et al.* (1999) proved that heat treatment doubled the levels of β-damascenone in wines, but enzyme treatment generated half the levels of this compound. Schneider *et al.* (2001), found that 1,6,6-trimethyl-1,2- dihydronaphtalene and β-damascenone were released from glycosidic extracts of Muscadet wines under mild acid conditions. Kovats (1987) proved that the compound mainly responsible for the sweet odour of Bulgarian oil of rose was shown to be a dehydroisoionone comprising 0.1% of the oil. Because of its organoleptic importance, damascenone is proposed as common name for this substance. The presence of damascenone and related compounds in the volatile oil of *M.unguis-cati* is of particular significance. Damacenone is present in *M.unguis-cati (*0.76%, of the oil) giving its leaves a sweet odour.

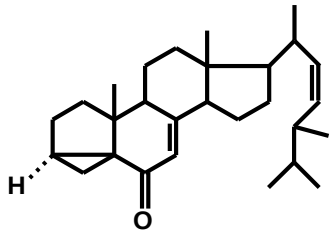
Table 2. GC/MS of unsaponifiable matter of the petroleum ether extract of *M.unguis-cati.*

No.	Compound	Mol. Formula	Mol. weight	Bp	Relative. area perc.
1	3-Ethoxy-1-propanol.	$C_5H_{12}O$	104	43	1.90
2	Ethyl ether	$C_4H_{10}O$	74	45	0.22
3	Cyclohexanone	$C_6H_{10}O$	98	55	0.99
4	2-Butanone,3-hydroxy	$C_4H_8O_2$	88	45	0.80
5	Decane	$C_{10}H_{22}$	142	57	0.33
6	1-Propoxy-2-propanol	$C_6H_{14}O_2$	118	45	0.12
7	2-Ethyl hexanol	$C_8H_{18}O$	130	57	4.91
8	3-Methyl-2-heptanol	$C_8H_{18}O$	130	45	0.05
9	Undecane	$C_{11}H_{24}$	156	57	1.28
10	2-Methyl undecane	$C_{12}H_{26}$	170	43	0.25
11	Cyclohexanol,5-methyl-2-(1-methylethyl)	$C_{10}H_{20}O$	156	71	0.22
12	1-Dodecene	$C_{12}H_{24}$	168	55	0.14
13	Dodecane	$C_{12}H_{26}$	170	57	1.48
14	2,6-dimthyl undecane	$C_{13}H_{28}$	184	57	0.23
15	Dodecane, 2-methyl	$C_{13}H_{28}$	184	43	0.07
16	Dodecane, 4, 6-dimethyl	$C_{14}H_{30}$	198	57	0.13
17	Tridecane	$C_{13}H_{28}$	184	57	0.33
18	Tetradecene	$C_{14}H_{28}$	196	43	0.73
19	1-Octanol, 2-butyl	$C_{12}H_{26}O$	186	57	0.32
20	2,5-Di-tert-amylquinone	$C_{16}H_{24}O$	248	57	0.58
21	6-(2hydroxypropyl)-2,2-dimethyl benzofuran-4,7 (2H,3H)-dione	$C_{13}H_{16}O_4$	236	177	0.13
22	Pentadecane, 2,6, 10-trimethyl	$C_{18}H_{38}$	254	57	0.57
23	Butylated hydroxyl toluene	$C_{15}H_{24}O$	220	205	11.97
24	2(4H)-benzofuranone 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (Dihydroactinidiolide)	$C_{11}H_{16}O_2$	180	111	1.00
25	1-Hexadecanol (cetal)	$C_{16}H_{34}O$	242	55	2.59
26	Undecane, 5-phenyl	$C_{17}H_{28}$	232	91	0.06
27	Nonylphenol isomer	$C_{15}H_{24}O$	220	121	4.4
28	Neophytadiene	$C_{20}H_{38}$	278	68	16.84
29	1-Eicosyne	$C_{20}H_{38}$	278	82	31.35
30	Isophytol	$C_{20}H_{40}O$	296	71	1.42
31	Phytol	$C_{20}H_{40}O$	296	71	1.38
32	Docosane	$C_{22}H_{46}$	310	57	0.19
33	Bis (2-ethyl hexyl) phthalate	$C_{24}H_{38}O_4$	390	149	2.21
34	3 α`, 5-cyclo-Ergosta-7,22-dien-6-one	$C_{28}H_{42}O$	394	394	0.80
35	Squalene	$C_{30}H_{50}$	410	69	1.13
36	β-sitosterol	$C_{29}H_{50}O$	414	43	1.30
37	β-amyrin	$C_{30}H_{50}O$	426	207	0.84

Composition of unsaponifiable fraction and fatty acids from petroleum ether extract

GC/MS of the unsaponifiable fraction revealed 37 compounds, (Table 2), some of them were identified in GC/MS of the volatile components. It is also characterized by high amount of oxygenated compounds. Analyses of fatty acids as the methyl esters enabled the identification of 21 fatty acids, representing 83.16% (Table 3). It is noteworthy to mention that the unsaponifiable fraction of *M.unguis-cati* is characterized by high percentage of oxygenated compounds and low percentage of hydrocarbons as it is the case with the volatiles.

Two triterpenoids were identified by GC/MS analysis of the unsaponifiable fraction, namely: β-amyrin $C_{30}H_{50}O$ with MW= 426 and base peak m/z 207, identity being confirmed by comparison with published data (Devon and Scott, 1972). The other triterpene was identified as squalene ($C_{30}H_{50}$) with MW= 410 and base peak m/z 69. -sitosterol with molecular formula $C_{29}H_{50}O$ and MW 414 was identified. In addition, another steroidal compound was identified as 3 ά,5 – cyclo- ergosta -7, 22- dien-6-one with M^+394 and molecular formula $C_{28}H_{42}O$, base peak at m/z 394.



3 ά, 5-cyclo-ergosta- 7,22 – dien-6-one

Table 3. Fatty acids(identified as methyl esters) of aerial part of *M.unguis-cati.*

No.	Fatty acid	Mol. Formula	Mol. Weight	BP	Relative area%
1	Octanoic acid	$C_9H_{18}O_2$	158	74	0.39
2	Dodecanoic acid	$C_{13}H_{26}O_2$	214	74	0.41
3	Tetradecanoic acid	$C_{15}H_{30}O_2$	242	74	2.43
4	Pentadecenoic acid	$C_{16}H_{30}O_2$	254	96	1.14
5	Pentadecanoic acid, 14- methyl	$C_{17}H_{34}O_2$	270	74	31.82
6	11-hexadecenoic acid	$C_{17}H_{32}O_2$	268	55	2.64
7	Heptadecanoic acid, 14 methyl	$C_{18}H_{36}O_2$	284	74	0.41
8	Hexadecanoic acid,14-methyl	$C_{18}H_{36}O_2$	284	74	0.59
9	Heptadecanoic acid,16-methyl	$C_{19}H_{38}O_2$	298	74	2.37
10	9,12-octadecadienoic acid, (Z,Z)	$C_{19}H_{34}O_2$	294	67	5.72
11	9,12,15- octadecatrienoic acid (Z,Z,Z).	$C_{19}H_{32}O_2$	292	79	19.72
12	6- tridecenoic acid, 13-(2-cyclopenten-1-yl,)	$C_{19}H_{32}O_2$	292	67	0.83
13	Eicosanoic acid	$C_{21}H_{42}O_2$	326	74	4.98
14	Heneicosanoic acid	$C_{22}H_{44}O_2$	340	74	1.07
15	Docosanoic acid	$C_{23}H_{46}O_2$	354	74	3.91
16	Tricosanoic acid	$C_{24}H_{48}O_2$	368	74	0.58
17	Tetracosanoic acid	$C_{25}H_{50}O_2$	382	74	1.89
18	Pentacosanoic acid	$C_{26}H_{52}O_2$	396	74	0.51
19	Hexacosanoic acid	$C_{27}H_{54}O_2$	410	74	0.55
20	Octacosanoic acid	$C_{29}H_{58}O_2$	438	74	0.79
21	Triacontanoic acid	$C_{31}H_{26}O_2$	466	74	0.41

LD_{50} of different extracts of *M.unguis-cati*

Study of LD_{50} of the different extracts of *M.unguis-cati* revealed that oral administration of 4.9 g/kg total alcohol extract killed 50% of the tested animals, while that of petroleum ether extract, being 4.5 g/kg (Table 4).

Table 4. LD_{50} of different extracts of *M.unguis-cati.*

Extract	LD_{50} (g/kg b.wt.)
Total alcohol extract	4.9
Petroleum ether extract	4.5
Chloroform extract	3.2
Ethyl acetate extract	3.1
Coumarin fraction	3.7

Analgesic and antipyretic activities of different extracts of *M.unguis-cati*

From tables 5 and 6, it can be concluded that the most potent extract of *M.unguis-cati* as antipyretic and analgesic is the total ethanol extract, the highest analgesic activity is obtained after one hour (69.5%), decreasing after the second hour to 68.8%. Antipyretic activity was exhibited after two hours from total ethanol, and ethyl acetate extracts 58.2% and 52.2%, respectively. The analgesic and antipyretic activities may be attributed to the phenolic compounds present in the ethanol extract.

Table 5. Antipyretic activity of successive extracts of *Macfadyena unguis-cati.*

Group*	Dose in mg/kg b.wt.	Induced rise in temperature	Body temperature change					
			One hour			Two hours		
			Mean ± S.E**	%of change	Potency after one hour.	Mean ± S.E**	%of change	Potency after two hours
Control	1mL saline	38.9 ± 0.2	38.7 ± 0.3	--	--	39.1 ± 0.4	--	-
Pet. ether	100	38.8 ± 0.2	38.2 ± 0.2	1.55	26.6	37.9 ± 0.3	2.3	29.3
Chloroform	100	39.2 ± 0.2	38.4 ± 0.3	2.04	35.1	37.8 ± 0.3	3.57	45.5
Ethyl acetate	100	39.1 ± 0.4	38.3 ± 0.3	2.04	35.1	37.5 ± 0.2	4.09	52.2
Total ethanol	100	39.2 ± 0.1	38.1 ± 0.1	2.8	48.1	37.4 ± 0.2	4.56	58.2
Coumarin fraction	100	38.7 ± 0.3	38.1 ± 0.4	1.55	26.6	37.6 ± 0.1	2.84	36.2
Paracetamol	20	39.5 ± 0.4	37.2 ± 0.2	5.82	100	36. 4± 0.1	7.84	100

*Albino rats (n=6)

**Significantly different from zero time at P <0.01 % of change calculated as regard zero time.

Table 6. Analgesic activity of successive extracts of *Macfadyena unguis-cati.*

Group	Dose in mg/kg b.wt.	Volts needed before treatment zero time	Volts needed after single oral dose					
			One hour			Two hours		
			Mean ± S.E	%of change*	Potency after one hour.	Mean ± S.E	%of change*	Potency after two hours
Control	1mL saline	72.3 ± 1.9	73.1 ± 1.4	--	--	72.9 ± 1.6	--	--
Petroleum ether	100	73.8 ± 1.5	104.2 ± 3.8	41.2	36.5	119.5 ± 3.8	61.9	47.1
Chloroform	100	76.4 ± 1.3	106.8 ± 3.9	39.79	35.3	121.2 ± 4.7	58.6	44.6
Ethyl acetate	100	75.2 ± 1.6	109.6 ± 4.1	45.7	40.5	113.2 ± 5.1	50.5	38.5
Total ethanol	100	71.6 ± 1.4	127.8 ± 4.2	78.4	69.5	136.3 ± 4.4	90.3	68.8
Coumarin fraction	100	74.5 ± 1.2	118.3 ± 4.5	58.79	52.1	127.2 ± 5.3	70.7	53.8
Dipyrone metamizol	50	77.2 ± 1.7	164.3 ± 5.9	112.8	100	178.6 ± 6.1	131.3	100

*Significantly different from zero time at P <0.01 % of change calculated with reference zero time.

REFERENCES

Adams, R.P. (1995). *Identification of Essential Oil Components by Gas Chromatography Mass Spectroscopy*. Allured Publishing Corporation, Carol Stream. Illinois USA.

Adriani, C., C. Lavarone, and C. Trogolo (1982). 5,7-Bisdeoxy-cynanchoside, an iridoid glucoside from *Macfacdyena cynanchoides. Phytochemistry*, 21: 231-233.

Bianco, A.D., M. Guiso, C. Lavarone and C. Trogolo (1974). Iridoids XV. Macfadienoside structure and configuration. *Gazzeta Chimica Italiana* , 104: 731-738.

Bolt A., S. Purkis and J. Sadd (1983). A damascone derivative from *Nicotiana tabacum*. *Phytochemistry*, 22: 613-614.

Bonini, C, E. Davini, C. Ivarone and C. Trogolo (1981). Cynanchoside a highly oxygenated iridoid glucoside from *Macfadyena cynanchoides. Phytochemistry*, 20: 1587-1590.

Bush, J.E. and R.W. Alexander(1960). An improved method for the assay of anti-inflammatory substances in rats. *Acta Endocrinologica,* 35: 268-276.

Cahoon, EB, S. Shah, J. Shanklin and J. Browse (1998). A determinant of substrate specificity predicted from the acyl-acyl carrier proteindesaturase of developing cat's claw seed *Plant Physiology*, 117: 593-598

Charlier, R., H. Prost, F. Binon, and G. Dellous, (1961). Pharmacology of an antitussive, 1-phenethyl-4 (2-propenyl) -4-propinoxypipridine acid fumarate. *Arch. Intern. Pharmacodynamic*, 134: 306-327.

Devon, TK. and A.I. Scott (1972). *Naturally occurring compounds* Vol. II. Academic press. New York, London.

Duarte, DS., M.F. Dolabela, C.F. Salas, D.S. Raslan, A.B. Oliveiras, A. Nenninger, B. Wiedemann, H. Wagner, J. Lombardi, and M.T.P. Lopes (2000). Chemical characterization and biological activity of *Macfadyena unguis-cati* (*Bignoniaceae*). *J. Pharm. Pharmacol*, 52: 347-352.

Ferrari, F., K.I. Cornelio, F. Delle Monache, and G.B. Marini Bettolo (1981). Quinovic acid glycosides from roots of *Macfadyena unguis-cati*. *Planta Med*., 43: 24-27.

Fournet, A., A.A. Barrios, and V. Munoz (1994). Leishmanicidal and Trypanocidal activities of Bolivian medicinal plants. *J. Ethnopharmacol.*, 41: 91-97.

Graletto. L. (1995). Nectary structure and nectar characteristics in some *Bignoniaceae. Plant systematics and evolution*, 1-2 : 99-121.

Houghton, PJ. and I.M. Osibogun (1993). Flowering plants used against snakebite. *J.Ethnopharmacol.* 39: 1-29.

Joshi, KC., P. Singh and M.C. Sharma (1985). quinones and other constituents of *Markhamia platycalyx* and *Bignonia unguis-cati*. *J. Nat. Prod*., 48: 145.

Kodama H., T. Fujimori and K. Kato (1984). Glucosides of ionone – related compounds in several *Nicotiana* species. *Phytochemistry*, 23: 583-585.

König, WA., P. Evers, R. Krebber, S. Schulz, C. Fehr, and G. Ohloff (1989). Determination of the absolute configuration of α-damascone and α-ionone from black tea by enantioselective capillary gas chromatography. *Tetrahedron*, 45: 7003-7006.

Kotseridis Y., R. Baumes and G. Skouroumounis (1999). Quantitative determination of free and hydrolytically liberated β-damascenone in red grapes and wines using a stable isotope dilution assay. *J. Chromatogr.*, A, 849: 245-254.

Kovats E. (1987). Composition of essential oils. Part 7. Bulgarian oil of rose (*Rosa damascena* mill.) *J. Chromatogr.*, A, 406: 185-222.

Miller LC. and M.I. Tainter (1944). Estimation of the LD_{50} and its error by means of logarithmic probit graph paper. *Proc. Soc. Expt. Biol and Med*., 57: 261-264.

Paget, G. and S. E. Berne' (1964). Toxicity tests in evaluation of drug activities. In: *The laboratory rat* (Laurence, DR. and Bacharach, AL eds), pp.135-160.. Academic Press London.

PioCorrea, M. (1978). Dicionario das plantas lcis do Brasil e das Exoticas cultivadas. Zmprensa Nacional, Ministerio da Agricultura, IBDF, *Rio de Janeiro, Brasil* ,6: 1926-1954.

Poser G., J. Schripsema, A. Henriques and S. Jensen (2000). The distribution of iridoids in *Bignoniaceae. Biochem. Syst. Ecol*., 28: 351-366.

Schneider R., A. Razungles, C. Augier and R. Baumes (2001). Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. *J. Chromatogr.* A, 936: 145-157.

Snedecor, WG. and G.W. Cochran (1982). *Statistical methods* 10th ed., Iowa state, University Press, USA.

Subramanian S., S. Nagarajan and N. Sulochana (1972). Flavonoids of eight bignoniaceous plants. *Phytochemistry*, 11: 1499.

Werkhoff, P., W. Bretschneider, M. Guntert, R. Hopp and H. Swburg (1991), Chirospecific analysis in flavor and essential oil chemistry part B. Direct enantiomer resolution of *trans*-α-ionone and *trans*-α-damascone by inclusion gas chromatography. *European Food Research and Technology*, 192: 111-115.

Williams P., C. Strauss and B. Wilson (1982). Use of C18 reversed – phase liquid chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *J. Chromatogr.* A, 235: 741-480.

(*Accepted for publication October 2006*)