Antioxidant study of flavonoid derivatives from the aerial parts of *Rhus natalensis* growing in Saudi Arabia

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Abstract: Phytochemical study of the CH_2Cl_2 soluble fraction of the aerial parts of *R. natalensis* resulted in the isolation and identification of six flavonoid derivatives, β-amyrin and β-sitosterol glucoside (daucosterol). The isolated compounds were identified utilizing physical, chemical and different spectral methods including UV, 1D- 2D-NMR and MS. The compounds were identified as four flavanones; 7-O-methyl hesperetin (1), 7-O-methyl naringenin (4), (-)-homoeriodictyol (eriodictyonone) (5), eriodictyol-7-methyl ether (6) and two flavones; 7-O-methyl isokaemferide (2) and genkwanin (3). The isolated compounds as well as some available standards representing structurally similar flavones and flavanones were used to study their antioxidant potential using DPPH and try to explore the impact of structures on the antioxidant activity. In other assays flavanones were less active than flavones as antioxidant due to lack of the C-4 carbonyl group in conjugation with 2,3-double bond. However, in DPPH assay based on the ability of molecules to donate hydrogen flavanones were found more active than flavones.

Keywords: *Rhus natalensis*; flavanones; flavones; DPPH; antioxidant.

INTRODUCTION

Genus *Rhus* is presented in Saudi Arabia by three species (Collenette, 1999). Several folk medicinal uses in African countries were reported for *Rhus natalensis*. In Kenya and Tanzania the roots of *R. natalensis* are used for treatment of hernia and stomach ache (Geissler, *et al*., 2002; Kamuhabwa, *et al*., 2000). The roots are also used in Kenya for treatment of venereal diseases, heartburn, cold, cough and diarrhea (Jeruto, *et al*., 2008). The infusion prepared from the leaves is used in Uganda for treatment of syphilis (Ssegawa, *et al*., 2007). In South African medicine the roots are used to treat fits in children (Stafford, *et al*., 2008). Combination of *Rhus natalensis* and *Turraea robusta*; *Rhus natalensis* and *Boscia salicifolia* exhibited high malarial parasite suppression when tested in mice (Gathirwa, *et al*., 2008). Previous Phytochemical study of the polar fractions of *Rhus natalensis* growing in Saudi Arabia resulted in the isolation of two chalcone glucoside, 3-methoxy neosakuranin, neosakuranin and luteolin.

Flavonoids are well known with antioxidant properties that may be responsible for their various biological activities (Havsteen, 1983, Brandi, 1992). Consumption of fresh fruits and vegetables help in decrease the incidence of cancer (Ingram *et al.,* 1997), heart disease (Frei, 1995) and stroke (Peterson and Dwyer, 1998) as indicated by epidemiological studies. This protective effect was attributed to the flavonoid contents based on several *in vitro*, *ex vivo* and animal studies, (Gorinstein *et al.,* 1998), few epidemiological and human studies (Knekt *et al.,* 1997; Hertog and Katan, 1998). Flavonoids exert their antioxidant activity through inhibition of enzymes generating reactive oxygen species; restore the levels of cellular defensive antioxidant species, scavenging the generated free radicals and chelating the elements involved in their production (Halliwell and Gutteridge, 2001).

In this work we report on the isolation of flavonoid derivatives from the less polar fraction and study their antioxidant activity using DPPH asay.

MATERIALS AND METHODS

General

Melting points were determined in open capillary tubes using Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus, and were uncorrected. Ultraviolet absorption spectra were obtained in methanol and with different shift reagents on a Unicum Heyios α UV-Visible spectrophotometer. Optical rotations were recorded on a Jasco P-2000 Polarimeter. CD were measured using J-815 CD Spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a UltraShield Plus 500MHz (Bruker) (NMR Unite at the College of Pharmacy, Salman Bin Abdulaziz University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, the coupling constants (*J*)

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are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard *Bruker* program. EI-MS were obtained using Finnegan MAT 300 mass spectrometer. ESIMS were obtained using Liquid Chromatography/Mass Spectrometer (Quattro micro API) equipped with a Z-spray electrospray ion source (Micromass®, Quattro micro™, WATERS). Silica gel 60/230-400 mesh (EM Science) and Sephadex® LH-20 (Pharmacia Fine Chemicals) were used for column chromatography, while silica gel 60 F254 (Merck) was used for TLC. Centrifugal preparative TLC (CPTLC) using Chromatotron (Harrison Research Inc. model 7924) and 4 mm silica gel P254 disc. PTLC was performed on pre-coated RP-18 F_{254} plates (MERCK).

Plant material

The plants of Rhus natalensis Bernh. ex Krauss were collected in March 2009 from Saqar, Abha, southern Saudi Arabia. The plant was identified by Dr. M. Atiqur Rahman, Prof. of Taxonomy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#14339) was deposited at the herbarium of the RCMAPP at College Pharmacy.

Extraction and isolation

The air-dried aerial parts of *R. natalensis* (1kg) were exhaustedly extracted with 90% ethanol (10L) at room temperature. The ethanol extract was evaporated under vacuum to yield 115g of dark green residue. The residue was dissolved in MeOH/H_2O (9:1) and defatted with hexane (3 X 400mL). The ratio of water was then increased to 20% and fractionated with CH_2Cl_2 (3 X 500 mL) followed by EtOAc (3 X 300mL).

The CH_2Cl_2 soluble fraction was evaporated under reduced pressure to give 23gm dry extract. Part of the CH_2Cl_2 soluble fraction (15gm) was chromatographed over silica gel column (450gm, 7cm i.d.) eluted with CH_2Cl_2/MeOH in a gradient system starting with 2% MeOH. Fractions 1- 3 (8gm) eluted with 2% MeOH in CH_2Cl_2 were further purified on another silica gel column eluted with 10% EtOAc in hexane with gradual increase in EtOAc contents.

Fractions eluted with 5% EtOAc in hexane (1.5gm) were again purified on silica gel column (60gm, 2cm i.d.) eluted with 5% EtOAc in hexane. Fractions 50ml each were collected examined by TLC and similar fractions were combined. Fractions 4-11 afforded 170mg of *β*-amyrin. Fractions 15- 20 afforded 130 mg of a mixture of two spots and were subjected to CPTLC (4mm silica gel disc) using 3% EtOAc in hexane to give 35mg of 1 and 21 mg of 2 upon crystallization from MeOH.

Fractions eluted with 10% EtOAc in hexane (1gm) were further chromatographed on silica gel column (40gm, 2 cm i.d.) eluted with 7% EtOAc in hexane. Fractions 50ml each were collected, screened by TLC and similar fractions were collected. Fractions 6- 10 afforded 23mg of 3 after crystallization from MeOH. Fractions 15- 18 afforded 18mg of 4 after repeated crystallization from MeOH.

Fractions eluted with 15% EtOAc in hexane (1.6 gm) were further chromatographed on silica gel column (60 gm, 2cm i.d.) eluted with 10% EtOAc in hexane. Fractions of 50ml were collected, examined by TLC and similar fractions were combined. Fractions 5- 11 afforded 20 mg of 5 after crystallization from MeOH. Fractions 20-27 afforded 31mg of 6 after repeated crystallization from MeOH.

Fractions eluted with 20% EtOAc in hexane (2 gm) were crystallized from MeOH to afford 179 mg of *β*-sitosterol glucoside.

Extraction and isolation procedures are presented in Scheme 1.

In vitro antioxidant activity using DPPH radical Scavenging assay

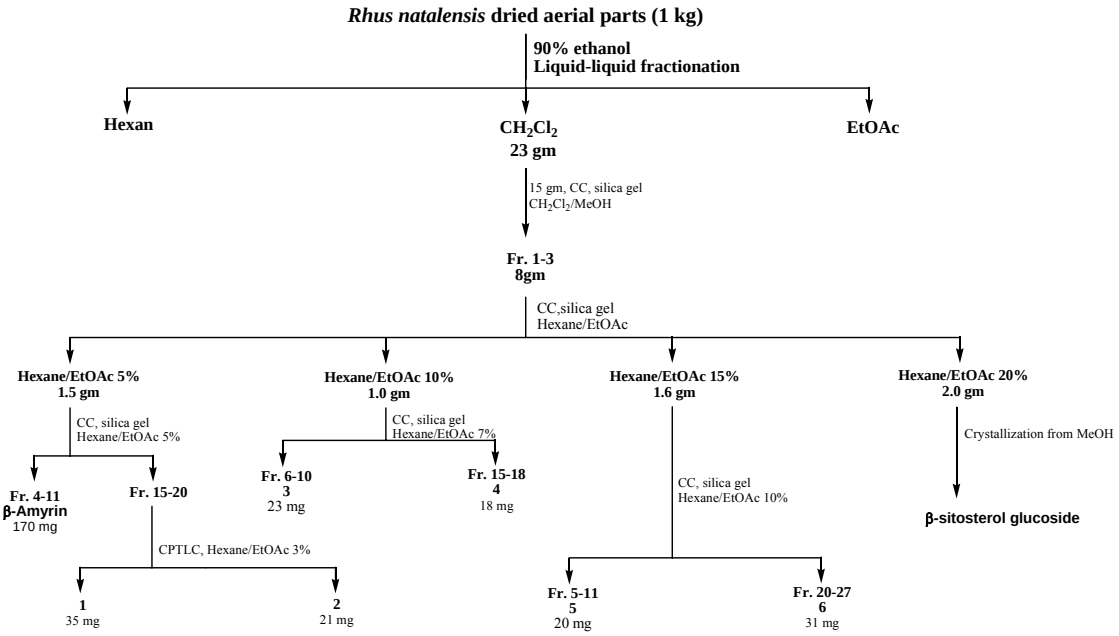
The experiment was preformed as described by Brand *et al*., 1995. Various concentrations (10. 50, 100, 500 and 1000µg/ml) of the crude extract and fractions were used. The assay mixtures with 1 ml total volume composed of 500µL of the extract, 125 µL prepared DPPH and 375µL solvent. Ascorbic acid was used as the positive control. The mixtures were incubated for 30 min at 25°C and the decrease in absorbance was measured at λ=517 nm. The radical scavenging activity was calculated from the equation:

% radical scavenging activity= $((A_{control} - A_{sample})/ A_{control})$ X 100

RESULTS

Phytochemical investigation of the CH_2Cl_2 soluble fraction afforded four flavanones (1, 4-6), two flavones (2, 3), *β*-amyrin and *β*-sitosterol glucoside. ^{1}H- and ^{13}C-NMR data of compounds 1- 6 are presented in tables 1 and 2. Comparative antioxidant study of the isolated compounds, diosmetin, diosmin, hesperidin and rutin were conducted aiming to correlate activity to structure variations. Results are presented in table 3.

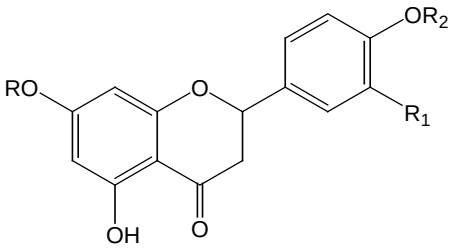
7-O-methyl hesperetin (5,3´,4´-trihydroxy-7-methoxyflavanone)(1): M.P. 283-285°C. $[\alpha]_D$ = -6 (*c*=0.016, MeOH). CD (*c* = 0.010, MeOH) Δε: 332 (1.0), 310 (0.0), 293 (-3.74), 262 (0.0). UV λ_{max} (MeOH) 330 (sh), 286; (NaOMe) 353 (sh), 287, 243; ($AlCl_3$) 377 (sh), 309, 225; ($AlCl_3$/HCl) 377 (sh), 308, 224; (NaOAc) 330 (sh), 286, 218 nm. ^{1}H- and ^{13}C-NMR (C_6D_6): tables 1 and 2. EIMS: 316 (17, M^+), 281 (29), 207 ($C_{14}H_7O_2$,100), 167 ($C_8H_7O_4$,



Scheme 1: Schematic presentation of the extraction and isolation procedures of *R. natalensis* compounds.

54), 150 ($C_9H_{10}O_2$, 39), 137 ($C_8H_9O_2$, 45), 123 ($C_7H_7O_2$, 14), 105 (C_7H_6O, 11), 73 (78).

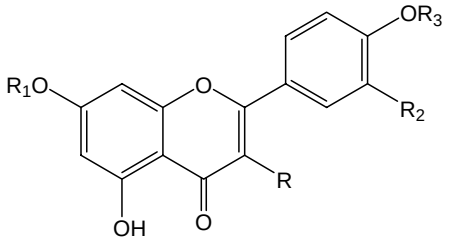
Jaranol (kaempfrol 3, 7-dimethyl ether, Kumatakenin) (2): M.P. 247-248°C. UV λ_{max} (MeOH) 345, 268, 250; (NaOMe) 405, 232 (sh), 260; ($AlCl_3$) 388, 361, 295 (sh), 275, 263; ($AlCl_3$/HCl) 388, 357, 297 (sh), 278, 262; (NaOAc) 405, 355, 264 nm. ^{1}H- and ^{13}C-NMR (DMSO-d_6): tables 1 and 2. ESIMS: 337 (100, $[M + Na]^+$).



	R	R_1	R_2
1:	CH_3	OH	CH_3
4:	CH_3	H	H
5:	H	OH	CH_3
6:	CH_3	OH	H
Hesperidin:	Rutinose	OH	CH_3

Genkwanin (3): M.P. 295-297°C. UV λ_{max} (MeOH) 335, 268; (NaOMe) 388, 267, 254; ($AlCl_3$) 380, 346, 301, 276; ($AlCl_3$/HCl) 380, 343, 300, 277; (NaOAc) 389, 295 (sh), 267 nm.^{1}H- and ^{13}C-NMR (DMSO-d_6): tables 1 and 2. ESIMS: 307 (100, $[M + Na]^+$).

7-O-Methyl naringenin (sakuranetin)(4): M.P. 154°C. $[\alpha]_D$ =2.5 (*c*=0.009, MeOH). CD (*c*=0.010, MeOH) Δε: 329 (17.6), 308 (0.0), 287 (-61.77), 262 (0.0), 215 (69.41). UV λ_{max} (MeOH) 318 (sh), 287; (NaOMe) 358 (sh), 287, 243; ($AlCl_3$) 377 (sh), 310, 225; ($AlCl_3$/HCl) 378 (sh), 309, 225; (NaOAc) 319 (sh), 286 nm. ^{1}H- and ^{13}C-NMR ($CDCl_3$): tables 1 and 2. EIMS: 286 (72, M^+), 207 (52), 167 ($C_8H_7O_4$, 100), 166 ($C_8H_6O_4$, 33), 138 ($C_7H_6O_3$, 39), 120 (C_8H_8O, 38), 107 ($C_6H_3O_2$, 12), 95 (45), 73 (37).



	R	R_1	R_2	R_3
2:	OCH_3	H	H	H
3:	H	H	H	H
Diosmetin:	H	H	OH	CH_3
Diosmin:	H	Rutinose	OH	CH_3
Rutin:	O-Rutinose	H	OH	H

(-)-Homoeriodictyol (eriodictyonone) (5): M.P. 223-225 °C. $[\alpha]_D$ = -4.5 (*c*=0.0073, MeOH). CD (*c*=0.012, MeOH) Δε: 328 (2.2), 309 (0.0), 290 (-5.4), 268 (0.0), 218 (7.22). UV λ_{max} (MeOH) 316 (sh), 289; 230, (NaOMe) 323, 248;

Table 1: ^{1}H-NMR chemical shift values* in ppm (multiplicity, *J* in parentheses in Hz) of 1- 6

No.	1[a]	2[b]	3[b]	4[c]	5[d]	6[b]
2	4.82_{ax} (dd, 12.5, 2.5)	-	-	5.37_{ax} (dd, 10.5, 2.5)	5.35_{ax} (dd, 13, 3)	5.4_{ax} (dd, 12.5, 2)
3	2.46_{eq} (dd, 17.5, 2.5) 2.69_{ax} (dd, 17.5, 12.5)	-	6.83 (s)	2.81_{eq} (dd, 17, 2.5) 3.11_{ax} (dd, 17, 10.5)	2.73_{eq} (dd, 17, 3) 3.14_{ax} (dd, 17, 13)	2.72_{eq} (dd, 17, 2) 3.24_{ax} (dd, 17, 12.5)
6	6.34 (d, 2.5)	6.36 (d, 2)	6.36 (d, 1.5)	6.06 (d, 2.5)	5.91 (d, 2.5)	6.08 (bs)
8	6.24 (d, 2.5)	6.78 (d, 2)	6.75 (d, 1.5)	6.09 (d, 2.5)	5.93 (d, 2.5)	6.10 (bs)
2′	6.68 (d, 2.5)	7.58 (d, 7.5)	7.94 (d, 7.5)	7.35 (d, 8.5)	7.09 (d, 2)	6.89 (bs)
3′	-	6.94 (d, 7.5)	6.93 (d, 7.5)	6.09 (d, 8.5)	-	-
5′	7.05 (d, 7.5)	6.94 (d, 7.5)	6.93 (d, 7.5)	6.09 (d, 8.5)	6.84 (d, 8.5)	6.76 (bs)
6′	6.66 (dd, 7.5, 2.5)	7.58 (d, 7.5)	7.94 (d, 7.5)	7.35 (d, 8.5)	6.94 (dd, 8.5, 2)	6.76 (bs)
OCH_3	7: 3.21 (s), 3′: 3.26 (s)	3: 3.91 (s), 7: 3.87 (s)	7: 3.86 (s)	7: 3.83 (s)	3′: 3.89 (s)	7: 3.79 (s)

Table 2: ^{13}C-NMR chemical shift values* in ppm of 1- 6

No.	1[a]	2[b]	3[b]	4[c]	5[d]	6[b]
2	79.38	150.89	164.06	78.95	80.65	78.60
3	43.57	138.08	102.95	43.16	44.14	42.11
4	196.18	181.90	181.85	196.06	197.73	196.84
5	165.29	157.21	161.18	164.14	165.17	163.16
6	95.24	97.92	97.89	95.14	97.14	94.54
7	168.29	165.09	165.09	168.06	168.44	167.39
8	94.55	92.64	92.62	94.28	96.25	93.74
9	163.32	161.15	157.19	162.92	164.83	162.79
10	103.75	104.66	104.64	103.16	103.39	102.60
1'	130.73	121.36	120.96	130.54	131.77	129.27
2'	109.05	120.43	128.48	127.92	111.44	114.32
3'	146.76	115.73	115.96	115.69	148.17	145.16
4'	146.90	163.95	161.37	156.22	149.15	145.71
5'	114.68	115.73	115.96	115.69	116.21	115.30
6'	119.98	120.43	128.48	127.92	120.56	117.93
OCH_3	7: 55.30, 3': 55.02	3: 55.99, 7: 55.96	7: 55.98	7: 55.65	3': 56.89	7: 55.83

*Assignments based on COSY, HSQC, HMBC and NOESY experiments.
[a]Spectra were measured in C_6D_6.
[b]Spectra were measured in DMSO.
[c]Spectra were measured in $CDCl_3$.
[d]Spectra were measured in CD_3OD.

($AlCl_3$) 375 (sh), 311, 223; ($AlCl_3$/HCl) 375 (sh), 309, 223; (NaOAc) 323, 288 nm. ^{1}H- and ^{13}C-NMR (CD_3OD): tables 1 and 2. EIMS: 302 (4, M^+), 269 ($C_{15}H_9O_5$, 27), 207 ($C_{14}H_7O_2$, 61), 160 ($C_{10}H_8O_2$, 58), 153 ($C_7H_5O_4$, 14), 147 ($C_9H_5O_2$, 5), 123 ($C_6H_5O_3$, 13), 79 (100).

Eriodictyol-7-methyl ether (6): M.P. 137- 138°C. $[\alpha]_D$ =- 5.7 (*c*=0.0067, MeOH). CD (*c*=0.011, MeOH) Δε: 332 (3.3), 312 (0.0), 291 (-13.77), 260 (0.0). UV λ_{max} (MeOH) 326 (sh), 287; (NaOMe) 350 (sh), 288; ($AlCl_3$) 381 (sh), 309; ($AlCl_3$/HCl) 381 (sh), 308; (NaOAc) 334 (sh), 287 nm. ^{1}H- and ^{13}C-NMR (DMSO): tables 1 and 2. EIMS: 302 (10, M^+), 269 (62), 175 (21), 166 ($C_8H_6O_4$, 4), 151 ($C_7H_3O_4$, 7), 136 ($C_6H_3O_3$, 18), 123 ($C_6H_3O_3$, 28), 122 ($C_7H_7O_2$, 5), 105 (C_7H_6O, 27), 94 (29).

DISCUSSION

The plants of *R. natalensis* were extracted with ethanol. The residue left after evaporation of the solvent was dissolved in EtOH/H_2O mixture and fractionated with hexane, CH_2Cl_2 and EtOAc. Purification of the CH_2Cl_2 fraction by chromatography afforded six flavonoid derivatives, *β*-amyrin and *β*-sitosterol glucoside. *β*-amyrin (Shashi and Asish, 1994; Wang, *et al.*, 2002) and *β*-sitosterol glucoside (daucosterol) (Lendl, *et al.*, 2005) were identified by direct comparison with reference compounds and literature data.

The UV major absorption band of 1, 4- 6 at 286-289 nm (experimental) as well as the NMR signals for OCH-CH_2-

Table 3: Free radical-scavenging activity (DPPH-assay) of 1-6, diosmetin, diosmin, hesperidin and rutin

Compd.	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
1	39.7	67.6	74.4	86.2	90.3
2	23.4	38.8	47.6	79.8	88.0
3	1.3	7.1	12.1	17.2	22.1
4	5.0	9.4	21.0	26.6	32.8
5	73.7	94.9	98.9	95.9	94.6
Diosmetin	4.6	26.0	27.6	47.4	56.4
6	23	53.4	75.4	94.9	97.8
Diosmin	11.6	19.7	18.9	18.8	42.2
Hesperidin	19.9	33.6	48.7	77.5	84.5
Rutin	64.3	88.6	87.2	88.1	90.6
Ascorbic acid	41.0	86.4	95.5	98.1	98.3

CO (tables 1, 2) assigned for C-2, C-3 and C-4 pointed out to a flavanone derivatives (Mabry *et al.,* 1970; Harborne *et al.,* 1975; Agrawal, 1989). In the ^{1}H-NMR of the four compounds H-2 appeared as dd with $J_{ax\text{-}ax}$ (10.5-13 Hz) and $J_{ax\text{-}eq}$ (2.5- 3 Hz)(table 1) indicating the axial orientation (Borges-Argáez *et al.*, 2005, Mabry *et al.,* 1970; Shakil, *et al.*, 2008). The CD spectra of the four compounds showed a positive Cotton effect around 328-332 nm and a negative one at 287-293 nm (experimental) supporting the (*2S*) configuration of 1, 4- 6 (Slade, *et al.*, 2005; Guo, *et al.*, 2012; Messi, *et al.*, 2012). Ring A showed two meta coupled protons in the ^{1}H-NMR spectra of the four compounds assigned for H-6 and H-8 indicating C-5 and C-7 substituted skeletons. Flavanones 1, 5 and 6 ^{1}H-NMR spectra showed an ABX system diagnostic for 3', 4'- substitutions. The ^{1}H- and ^{13}C-NMR spectra of 1 showed two methoxyl signals at δ_H 3.21 (s), δ_C 55.30 and δ_H 3.26 (s), δ_C 55.02ppm assigned for positions 7 and 3' respectively based in NOESY experiment, where δ_H 3.21 signal showed NOE effect with H-6 and H-8 at δ_H 6.34 and 6.24 ppm respectively. On the other hand the δ_H 3.26 signal showed NOE effect only with H-2' at δ_H 6.68 ppm. The data of 1 are consistent with those reported for 7-O-methyl hesperetin (Maatooq, *et al.,* 2002). Flavanones 5 and 6 showed one methoxyl signal in each. The methoxyl signal of 5 at δ_H 3.89 showed NOE effect with H-2' at δ_H 7.09 supporting C-3' methoxylation. Consequently, 5 was identified as (-)-homoeriodictyol (eriodictyonone)(Liu, *et al.,* 1992; Shetgiri and Rege, 2003; Torres, *et al.,* 1996). In 6 the methoxyl signal at δ_H 3.79 showed NOE effect with H-6 and H-8 at δ_H 6.08 and 6.10 ppm respectively supporting C-7 methoxylation. Based on the above discussion 6 was identified as eriodictyol-7-methyl ether (Morales, *et al.,* 2009; Torres, *et al.,* 1996; Stavri, *et al.,* 2005).

^{1}H-NMR of 4 showed two doublets each integrated for 2H at δ_H 6.09 and 7.35ppm diagnostic for *p*-substituted ring B. The methoxy signal at δ_H 3.83 were assigned to C-7 position as it showed NOE effect with H-6 and H-8 at δ_H 6.06 and 6.09ppm respectively. The data of 4 supported the structure identification as 7-O-Methyl naringenin (sakuranetin)(Modak, *et al.,* 2007; Ferheen, *et al.,* 2005; del Barrio, *et al.,* 2011).

^{1}H-NMR of 2 and 3 showed two doublets each integrated for 2H at δ_H 6.09 and 7.35ppm diagnostic for *p*-substituted ring B. Ring A in both compounds showed two *meta* couples doublets assigned for H-6 and H-8 (table 1). UV data with shift reagents indicated a free C-5 and C-4' OH groups. The UV bands of 2 in MeOH at 345 nm as well as the 13C-NMR signal at δ_C 138.08ppm were diagnostic for substituted flavonol. Both ^{1}H- and ^{13}C-NMR of 2 (tables 1, 2) indicated the presence of two methoxyl groups. The methoxyl at δ_H 3.87 (s), δ_C 55.96 ppm showed NOSY correlations with H-6 and H-8 at δ_H 6.36 and 6.78 ppm respectively proving its position at C-7. The other methoxyl at δ_H 3.91 (s), δ_C 55.99 ppm should be located at C-3. The absence of any NOE correlation was in favour of such assignment. Compound 2 was consequently, identified as kaempfrol 3, 7-dimethyl ether (kumatakenin)(Ali, *et al.,* 2006). UV data of 3 indicated a flavone with 7-substituted hydroxyl group (Mabry *et al.,* 1970; Harborne *et al.,* 1975). The ^{1}H-NMR singlet at δ_H 6.83 ppm with its correlated carbon at δ_C102.95 ppm were assigned for position 3 in a flavones skeleton. The methoxyl at δ_H 3.86 (s), δ_C 55.98ppm showed NOSY correlations with H-6 and H-8 at δ_H 6.36 and 6.75 ppm respectively further support its position at C-7. The data of 3 are identical with that reported for genkwanin (Lin *et al.,* 2001; Grande *et al.,* 1985).

Based on the results of half peak oxidation potentials, iron chelation and enzymatic lipid peroxidation assays the radical scavenging activity was correlated to certain structural features. The C-4 carbonyl function conjugated with 2,3-double bond, the 3',4'-dihydroxyl groups and the C-3 hydroxyl group were the most important for strong activity. Additional hydroxyl or methoxyl groups at positions 3, 5 and 7 of rings A and C seem to be less

important (Van Acker *et al.*, 1996; Pietta, 2000). According to these findings flavanones and falvanonols are much less effective as radical scavenging molecules. However, these classes are less oxidized that their corresponding flavones and flavonols and somehow should have more antioxidant activity. DDPH (1,1-diphenyl-2-picrylhydrazyl) is a unique stable free radical widely used to estimate antioxidant activity. The DPPH assay is based on the ability of any material with suspected antioxidant activity to donate hydrogen atom to the violet coloured free radical and convert it to pale yellow reduced form (Molyneux, 2004). We selected this assay to compare the antioxidant activity of the isolated flavanones, hesperidin with their closely related flavones (table 3). The only difference between diosmin and hesperidin is the absence of 2,3-double bond in the latter. Hesperidin showed approximately double the antioxidant activity of diosmin. The antioxidant potential of rutin exceeded that of hesperidin and that is clearly due to the presence of 3',4'-dihydroxyl groups in ring B. (-)-homoeriodictyol (eriodictyonone)(5) reached it maximum activity (98.9%) at 100µg/ml. It was much more active than the closely related favone diosmetin (27.6%). The maximum activity of diosmetin was 56.4% at 1000 µg/ml. 7-O-Methyl naringenin (sakuranetin)(4) lacking 2,3-double bond was more active (32.8 % at 1000 µg/ml) than genkwanin (3) (22.1% at 1000 µg/ml) although both were weak due to the lack of 3'-substitution. Comparing the antioxidant potential of 7-O-methyl hesperetin (1) (90.3% at 1000 µg/ml) with that of naringenin (sakuranetin)(4) indicate the importance of ring B 3,4-dioxygenated substituent.

CONCLUSION

Phytochemical study of CH_2Cl_2 soluble fraction of the aerial parts of *R. natalensis* afforded two triterpenes, two flavones and 4 flavanones. The flavonoid derivatives were subjected to antioxidant activity using DDPH assay. The results proved that flavanones are more active that their related flavones in this assay. The presence of ring B 3,4-dioxygenated substituent make the compounds much more active. The results of our work clearly indicate that *in vito* antioxidant assay results are based on the mechanism of protection required in the assay.

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