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Study of the Chemical Composition of the Resinous Exudate Isolated from *Psoralea glandulosa* and Evaluation of the Antioxidant Properties of the Terpenoids and the Resin

[Estudio de la composición química del exudado resinoso aislado de *Psoralea glandulosa* y la evaluación de las propiedades antioxidantes de la resina y sus terpenoides]

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Abstract
Otholobium glandulosum (L.) J.W. Grimes (= *Psoralea glandulosa* L.) (Fabaceae) is a resinous bush that grows in Chile. The chemical composition of its resinous exudate was determined for the first time. Three meroterpenic: bakuchiol (**8**), 3-hydroxybakuchiol (**11**), 12-hydroxyisobakuchiol (**12**) and a new compound kuchiol (**13**) were isolated and their structures were determined. The antioxidant activity of the terpenic compounds and resin was evaluated using the bleaching of DPPH radical, Ferric Reducing Antioxidant Power (FRAP) and Total Reactive Antioxidant Properties (TRAP) assays.

Keywords: *Psoralea glandulosa*, antioxidant, resinous exudate, terpenoids, kuchiol

Resumen
Otholobium glandulosum (L.) J.W. Grimes (= *Psoralea glandulosa* L.) (Fabaceae) es un arbusto resinoso que crece en Chile. La composición química de su exudado resinoso fue determinada por primera vez. Tres meroterpenos: bakuchiol (**8**), 3-hidroxibakuchiol (**11**), 12-hidroxiisobakuchiol (**12**) y un nuevo compuesto kuchiol (**13**) fueron aislados y sus estructuras fueron determinadas espectroscópicamente. La actividad antioxidante de los compuestos terpénicos y la resina se evaluó mediante tres métodos antioxidantes DPPH, Poder Reductor de Hierro III (FRAP) y Capacidad Antioxidante Total (TRAP).

Palabras Clave: *Psoralea glandulosa*, exudado resinoso, antioxidantes, terpenoides, kuchiol

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INTRODUCTION

In recent years a worldwide increased production of free radicals and reactive oxygen species has been associated with a rise of a big number of pathologies such as cancer, AIDS, diabetes, inflammation and several cardiovascular diseases (Houghton *et al.*, 2007; Nickavar *et al.*, 2008). This had led to the necessary search for new antioxidant of natural origin. The medicinal plants represent a rich source of natural antioxidants (Halliwell *et al.*, 1995) and provide with new active molecules of potential use in medicine with fewer side effects, a wider spectrum of action and lower cost in current use (Mesa *et al.*, 2004). Among the candidates, the resinous exudates are known for possessing several biological activities, such as antioxidant and antimicrobial activities (Modak *et al.*, 2009; Faini *et al.*, 2011; Urzua *et al.*, 2012).

Otholobium glandulosum (L.) J.W. Grimes (= *Psoralea glandulosa* L.) (Fabaceae) is a medicinal herb used in Chilean, Peruvian and Bolivian folk medicine. It is known by the vernacular names "culen" and "cule" (Chile), "albaquilla del campo" (Perú), "villea" (Bolivia). *P. glandulosa* is commonly used in folk medicine for digestive disorders, especially diarrhea, wound healing and hemorrhoids. It acts as an anthelmintic, vulnerary, tonic and febrifuge. It is also known as a controversial hypoglycemic agent (Hoffmann *et al.*, 1992; Rozzi, 1984). The anti-inflammatory and antipyretic activities of the infusión and extracts of the aerial parts have been reported (Backhouse *et al.*, 2001).

The objective of this investigation is a chemical study of the resinous exudate isolated from *Psoralea glandulosa* and evaluation of the antioxidant properties of the three main terpenoids and the resin

EXPERIMENTAL SECTION

Plant material and extraction of the resinous exudates

Aerial parts of *P. glandulosa* L. were collected during the flowering season in Lo Orozco (V Region, Chile, 33º 13' S, 71º 25' W). A voucher specimen (Nº Pg-11123) was deposited in the Herbarium of the "Dr. Herbert Appel A.", Natural Products Laboratory, Department of Chemistry, Universidad Técnico Federico Santa María, Valparaíso, Chile.

The resinous exudate of *P. glandulosa* (10 g, 1.67%) was obtained by dipping fresh plant material (600 g) in cold CH_2Cl_2 for 25-30 s. at room temperature and the filtered solution was then concentrated under reduced pressure.

Analysis of Resinous Exudate

Gas Chromatography-Mass Spectrometry (GC-MS)

The resinous exudate was diluted with dichloromethane, 1 µL sample was analyzed by Gas Chromatography–Mass Spectrometry (GC/MS) using the instrumentation described above. Analysis was carried out using a Thermo Scientifics GC–MS system (GC: model Trace GC Ultra and MS: model ISQ) and operating in EI mode at 70 eV, equipped with a splitless injector (250 °C). The transfer line temperature was 250 °C. Helium was used as carrier gas at a rate of 1.3 mL/min, and the capillary column used was a Rtx-5ms (60 m × 0.25 mm i.d., film thickness 0.25 µm). The temperature program was 40 °C (5 min) to 250 °C (8 min) at a rate of 5 °C/min. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST08 library database, and by comparison of their retention index with those reported in the literature (Adams, 2007), for the same type of column or those of commercial standards, when available.

Spectroscopy Analysis

IR-FT spectra were recorded on a Nicolet 6700 spectrometer and frequencies were reported in cm^{-1}. ^{1}H, ^{13}C, ^{13}C DEPT-135, sel. gs 1D ^{1}H NOESY, gs 2D HSQC and gs 2D HMBC spectra were recorded in $CDCl_3$ solutions and were referenced to the residual peaks of $CHCl_3$ at δ = 7.26 ppm and δ = 77.0 ppm for ^{1}H and ^{13}C, respectively, on a Bruker Avance 400 Digital NMR spectrometer, operating at 400.1 MHz for 1H and 100.6 MHz for ^{13}C. ESI-MS/MS data was collected using a high resolution hybrid quadrupole (Q) and orthogonal time-of-flight (TOF) mass spectrometer (Micromass Q-Tof, Manchester, UK) with constant nebulizer temperature of 80 °C. The ESI source and the mass spectrometer were operated in a negative ion mode, and the cone and extractor potentials ware of 10 eV, with a scan range of *m/z* 100–500. The band infused into the ESI source at flow rates of 5 µL min^{-1} was dissolved in acetonitrile ion-induced dissociation (CID) with argon in the collision chamber. The values expressed are average mass and correspond to the [M-H].

Column chromatography separation of the resinous exudate

Part of the resinous exudates of *P. glandulosa* (2 g) was fractionated by CC (silica gel) using a Hexane-Ethyl acetate (EtOAc) of increasing polarity, afforded bakuchiol (**8**) (1402.6 mg) and impure fractions.

Further purification of impure fractions was achieved using a CC and preparative chromatography (silica gel Merck plates HF-254). The isolated compounds were as follows: 3-hydroxy-bakuchiol (**11**) (131.7 mg), 12-hydroxyisobakuchiol (**12**) (12.1 mg) and 4-[9-ethenyl-9,15-dimethyldeca-7,14-dienyl]-phenol (kuchiol) (**13**) (60.4 mg). The progress of separation of compounds was monitored and analyzed by TLC. TLC spots were detected by heating after spraying with 25% H_2SO_4 in H_2O.

Compound 13

Yellow oil. IR (cm^{-1}): 3346 (-OH), 1604, 965 (C=C), 3053, 1514, 1446, 1265, 1170, 895. ^1H NMR (400 MHz, $CDCl_3$) δ_H (H, m, *J* in Hz): 7.22 (H-2 and H-6, d, 8.4); 6.75 (H-3 and H-5, d, 8.4); 6.23 (H-7, d, 16.2); 6.03 (H-8, d, 16.3); 5.86 (H-19, dd, 10.8 and 17.3); 5.09 (H-14, t, 6.5); 5.00 (H-20, m); 1.93 (H-13, dd, 7.7 and 16.2); 1.65 (H-16, s); 1.56 (H-17, s); 1.47 (H-10, m); 1.23 (H-11 and H-12, m); 1.17 (H-18, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 154.7 (C-4); 145.9 (C-19); 135.7 (C-8); 131.3 (C-17); 130.8 (C-1); 127.3 (C-2 and C-6); 126.4 (C-7); 124.8 (C-14); 115.3 (C-3 and C-5); 111.8 (C-20); 42.5 (C-9); 41.2 (C-10); 29.7 (C-12); 29.4 (C-13); 25.7 (C-16); 23.3 (C-17); 23.2 (C-11); 17.6 (C-18). HREIMS: M+H ion m/z 285.4434 (calcd. For $C_{20}H_{28}O$: 284.4356).

Antioxidant Activity of the Resinous Exudate and the Major Compounds

Reagents for 2,2-diphenyl-1-picrylhidrazyl ($DPPH^{\bullet}$), $FeCl_3x6H_2O$, (2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), sodium acetate (NaOAc), acetic acid (HOAc), hydrochloric acid (HCl), Trolox™ 2,2'-azino-bis(3-ethylbenzothialzoline-6-sulfonic acid) ($ABTS^{*+}$) 2,2'-azo-bis(2-amidinopropane) (ABAP), phosphate buffered saline (PBS), α-tocopherol and Butylated hydroxyanisole (BHA) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and chemicals used were of analytical grade.

DPPH radical scavenging assay

The DPPH assay was performed as described previously by Brand-Williams *et al.* (1995) with modifications (Lakic *et al.*, 2010). 0.1 mL sample (from 0 to 4 mg/L of resin and 8, 11 and 13 compounds) was mixed with 2.9 mL $DPPH^{\bullet}$ solution (50 μM) and such a solution was freshly prepared in ethanol. 2.9 mL 50 μM $DPPH^{\bullet}$ solution with 0.1 mL ethanol was used as a control. The absorbance of the resulting solutions, control and the blank (with the reagents only) were recorded after 15 min at room temperature. Each sample was replicated three times. The disappearance of $DPPH^{\bullet}$ was detected spectrophotometrically at 517 nm. Percent RSC (Radical Scavenging Capacity) was calculated by the following equation:

$$\mathbf{RSC\ (\%) = 100\% \times (A_{control}\text{-}A_{sample})/\ A_{control}}$$

From the obtained RSC (%) values the IC_{50} value, which represent the concentrations of the resinous exudate and the major compounds that caused 50% inhibition, was determined by linear regression analysis.

Ferric Reducing Antioxidant Potential (FRAP) Assay

The ferric reducing power of the resinous exudate and the main compounds were measured as described by Dudonné *et al.* 2009 with modifications. The working FRAP reagent was daily prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. All solutions were used on the day of preparation. 100μL of sample solutions and 300 μL of deionized water were added to 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37 °C in a water bath. Then, the absorbance of the samples was measured at 593 nm. An ethanol blank reading was also taken. The difference between sample absorbance and blank absorbance was calculated and used to determine the FRAP value. FRAP values were expressed as mM Trolox™. All measurements were done in triplicate.

Total Reactive Antioxidant Properties (TRAP) assay

The method developed by Romay *et al.* (1996) was slightly modified in this experiment. A 10 mM solution of ABAP was mixed with 150 μM solution of $ABTS^{\bullet+}$ in 100 mM solution of PBS a pH 7.4. The mixture was incubated at 45 °C for 30 min. 10 μL of sample solution was added to 990 μL of the resulting blue-green $ABTS^{\bullet+}$ solution. The decrease of absorbance of TRAP solutions and $ABTS^{\bullet+}$ as blank were recorded after 30 s at room temperature. Then, the absorbance of the samples was measured at 734 nm. The total antioxidant capacity TRAP of extracts was expressed in mM Trolox™ equivalents (TEAC),

using a standard curve of Trolox™ (0-120 mg/L). All measurements were replicated three times.

Statistical analysis

The data were reported as mean values ± standard deviation (SD). Kruskal-Wallis ANOVA was used with a confidence level of 95%. Values representing the concentrations of investigated extracts that cause 50% of inhibition (IC_{50}) were determined by linear regression analysis of Radical Scavenging Capacity (%RSC); likewise FRAP and TRAP assays (STATISTICA 7.0 program).

RESULTS AND DISCUSSION

Analysis of the chemical composition of the resin

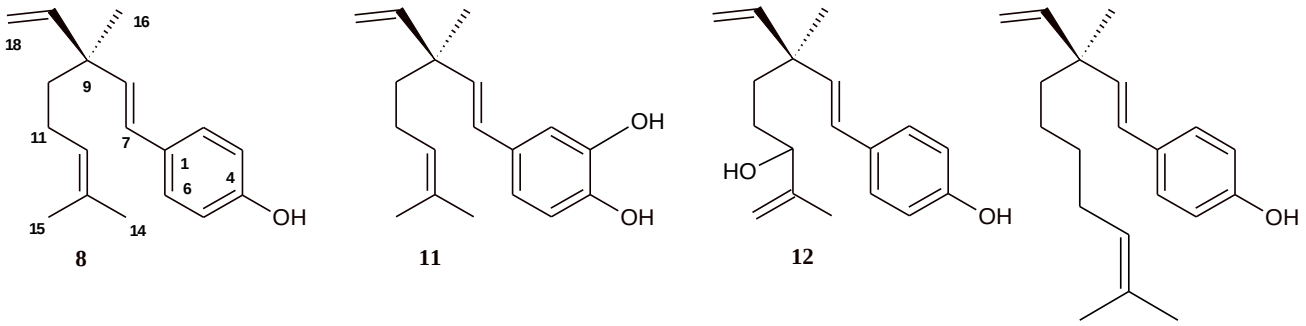
In this present work, the resinous exudate from leaves of *P. glandulosa* was analyzed by GC-MS and the isolated compounds were analyzed by IR, NMR and MS. The compounds characterized and reported with their relative percentages are listed in Table 1. In this study, 13 compounds were identified, representing 98.14% of the total resin, with oxygenated dominating terpenes (92.21%). The compounds **8**, **11** and **13** were present in the resin with a mass percentage of 69.4%, 6.5% and 3.0% respectively. The structural elucidation by NMR of four main secondary metabolites were isolated: three meroterpenes isolated, namely, bakuchiol (**8**), 3-hydroxybakuchiol (**11**) and 12-hydroxyisobakuchiol (**12**), plus a new compound that we have named kuchiol (**13**) (see Figure 1). Bakuchiol had been previously obtained from *Psoralea coryfolia, P. drupacea* and *P. graveolens* (Bondarenko *et al.*, 1977; Mehta *et al.*, 1973) and 3-hydroxybakuchiol and 12-hydroxyisobakuchiol have been found previously in the alcoholic extract of the leaves from *P. glandulosa* (Labbé *et al.*, 1996). Their structures were confirmed by comparison with authentic samples and with literature spectral data.

Table 1
Chemical composition of the resinous exudate from *Psoralea glandulosa* L.

N°	Compound	RI	% Area	Identification
1	Dodecane	1102	0.4	RI, MS, Co-I
2	Tridecane	1303	0.2	RI, MS, Co-I
3	Tetradecane	1400	0.2	RI, MS, Co-I
4	Pentadecane	1497	0.5	RI, MS, Co-I
5	Hexadecane	1599	0.2	RI, MS, Co-I
6	Caryophyllene oxide	1614	0.6	RI, MS
7	Octadecane	1799	0.3	RI, MS, Co-I
8	Bakuchiol	2139	64.0	RI, MS, Co-I, NMR
9	Cyclobakuchiol A	2194	3.5	RI, MS, Co-I
10	Cyclobakuchiol B	2257	3.9	RI, MS, Co-I
11	3-Hydroxybakuchiol	2401	9.6	RI, MS, Co-I, NMR
12	12-Hydroxyisobakuchiol	2438	4.5	RI, MS, Co-I, NMR
13	Kuchiol	2625	6.2	RI, MS, Co-I, NMR
	Unknown		6.0	

RI: Retention Index; MS: Mass spectra; Co-I: Co-injection; NMR: Nuclear Magnetic Resonance

Figure 1
Compounds isolated from resinous exudate obtained from *Psoralea glandulosa* L.



The compound **13** was characterized by its spectroscopic data. Compound **13** was obtained as yellow oil. The presence of hydroxyl group was evident from the IR absorption band at 3346 cm^{-1}. Also, the signal at 1604 and 925 cm^{-1} showed a system C=C. The ^{1}H NMR spectrum clearly showed that the aromatic ring, two signals (δ 6.23, d and 6.03, d) corresponding to the C-6, C-7 *trans* double bond, the vinyl group (δ 5.86, dd and 5.09, t), the hydroxyl group (δ 4.97, s), the C-18 methyl group (δ 1.17, s) and one signal 1.23 (m, 4H, H-11 and H-12) corresponding to two CH_2 group. The ^{13}C NMR spectrum presented signals for 20 carbons of which four are CH aromatics, one OH group united to aromatic ring and one attached to 14 carbons of the side chain. A DEPT experiment indicated the presence of five CH_2 confirming the presence of two carbons more than bakuchiol in the side chain. The assigned structure was confirmed by HREIMS M+H ion m/z 285.4434. This terpene was identified in the *Psoralea glandulosa* for the first time.

Antioxidant activity

The antioxidant activity of the terpenic compounds **8**, **11**, **13** and the resin was evaluated in a series of *in vitro* tests. Each of these assays is based on one feature of antioxidant activity, which is the ability to scavenge free radicals, reductive power and total radical-trapping power. The results of the antioxidant activities are summarized in Table 2.

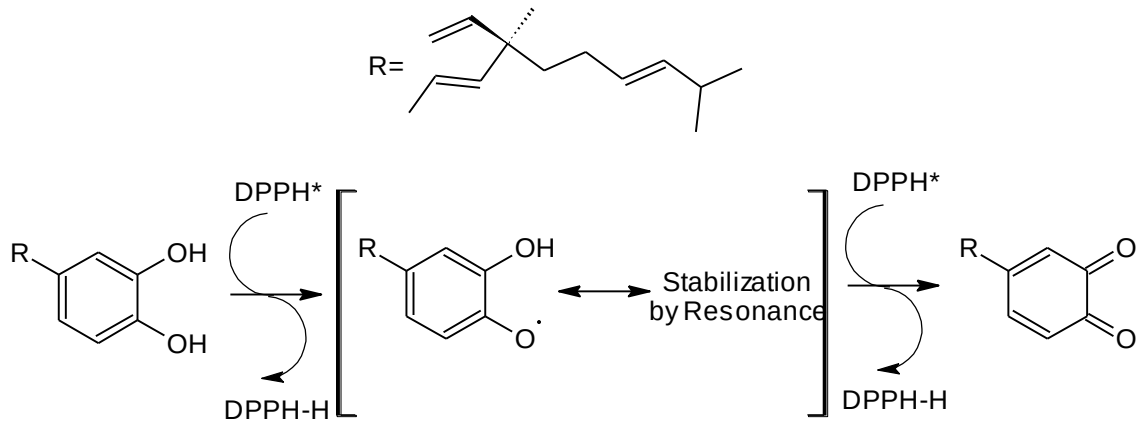
Table 2
Comparison of the antioxidant activity of compounds 8, 11, 13 and the resinous exudate from *Psoralea glandulosa*.

Compound	IC_{50} DPPH$^{\bullet}$ (μg/mL)	FRAP (mM Trolox ™)	TRAP (mM Trolox ™)
Resin	**0.76 ± 0.05**	**2.89 ± 0.01**	**1.21 ± 0.04**
8	**1.36 ± 0.08**	**2.96 ± 0.11**	**1.21 ± 0.03**
11	**0.34 ± 0.02**	**0.75 ± 0.01**	**1.12 ± 0.02**
13	**1.96 ± 0.04**	**3.12 ± 0.01**	**1.16 ± 0.03**
α-tocopherol	**6.54 ± 0.04**	**na**	**na**
BHA	**2.89 ± 0.02**	**na**	**na**

Values are given as mean ± S.D. of triplicate experiments.
BHA: butylated hydroxyanisole.
na: Not applicable

The IC_{50} values obtained by a DPPH assay for the compounds **8** and **11** (see table 2) showed that **8** had a minor radical scavenging capacity in comparison to **11** which presented an aromatic system with two hydroxyl groups in its structure. This difference of **11** compound gave a RSC four times higher than **8**. So, the feasibility of donating the $H^{\bullet}$ radical by means of hydroxyl groups of the aromatic system of **11** can be converted into *o*-quinone system (Brand-Williams *et al.*, 1995), see figure 2.

Figure 2. Mechanism of the regeneration of an ortho-diphenol



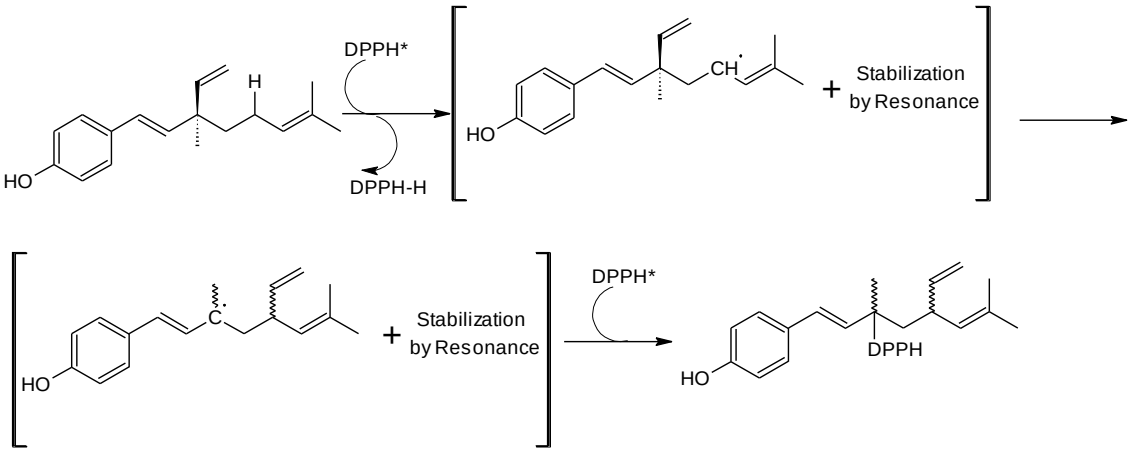
On the other hand, comparing the IC_{50} value of the compounds **8** and **13**, we could observe that **13** compound has two additional CH_2 groups in the lateral chain, causing a decrease in the antioxidant capacity (44%) with respect to the compound **8**. This can be explained by a mechanism shown in figure 3, where the allylic hydrogen in the alkyl chain of the test compounds, **8** is most labile and susceptible for abstraction by $DPPH^{\bullet}$. In comparison, the allylic radical adjacent to the trisubstituted olefin, which it could be formed by **8** or **11** but not by **13**, these results suggest that the compound **13** has less resonant structures. This is corroborated by synthesizing of the methyl ether of bakuchiol, with an IC_{50} value of 6.37 µg/mL. The lower inhibitory concentration of eter as compared to that of 8 and 11 was expected considering the lack of the easily oxidizable phenol group. The activity of eter can possibly be explained by abstraction of its allylic hydrogen by DPPH radical or addition of the radical to one of its olefinic functionalities (Adhikari *et al.*, 2003).

In the DPPH assay, it could be observed that the resinous exudate showed an IC_{50} of 0.76 µg/mL, so this value would explain a possible synergic effect of terpenphenols present into the resin (Shahidi, 2005).

FRAP and TRAP assays give information about the total antioxidant capacity (Denev *et al.*, 2010; Pellegrini *et al.*, 2003), indicating that the resin and the compound **8** have very similar values, matching with the proportionality of the compounds **8**, **11** and **13** into the resin. However, the main factor in FRAP and TRAP activities should be **8**. There is no correlation of results obtained with DPPH assay.

The antioxidant activities of natural compounds and resin and two control samples were evaluated using the same method. The compound **11** was much stronger than of the controls BHA and α-tocopherol. The antioxidant activities of the compounds decrease as follows: **11** > resin > **8** > **13** > BHA > α-tocopherol.

Figure 3.**Structure of the most stable radical**



This is the first report of its antioxidant effect in vitro. This type of molecules possesses the protective activity against oxidative damages to lipids and proteins (Adhikari *et al.*, 2003). It has been investigated and rationalized based on the scavenging activity against various oxidizing radicals ($CCl_3OO^{\bullet}$, $LOO^{\bullet}$, hydroxyl and glutathiyl radicals). The rate constants of the scavenging reactions, transients formed in these reactions and their mechanistic pathways had been probed using optical pulse radiolysis (PR) technique (Adhikari *et al.*, 2007). Another study was designed to evaluate the protective effect of methyl ether of bakuchiol against Fenton reaction induced lipid peroxidation in rat brain homogenate. The activity for this compound was explained by abstraction of allylic hydrogen by the lipid peroxyl radical ($LOO^{\bullet}$) (Adhikari *et al.*, 2003).

CONCLUSIONS

For the first time it has been reported the chemical composition of the resinous exudate of aerial parts concerning *Psoralea glandulosa*, where meroterpene bakuchiol was more dominant and new compound which was designated as kuchiol.

The resinous exudate presented an important antioxidant capacity in the DPPH assay mainly caused by the mixture of meroterpenic compounds. The isolated compound **11** had a higher RSC for DPPH assay.

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