*Malaysian Journal of Science* **25** (1): 45 – 51 (2006)

# Chemical Constituents and Biological Activities of *Glycosmis Chlorosperma* Var. *Elmeri* (Merr.) Stone (Rutaceae)

**Rosmiati Abdullah[1], Mawardi Rahmani[1,*], Abdul Manaf Ali[2], Hazar Bebe Mohd Ismail[3], Mohd Aspollah Sukari[1], Gwendoline Ee Cheng Lian[1]** and **Julius Kulip[4]**

[1] Department of Chemistry, Universiti Putra Malaysia, 43400 Serdang, Malaysia.
[*] mawardi@fsas.upm.edu.my. Facsimile (+ 603) 89435380
[2] Department of Biotechnology, Universiti Putra Malaysia, 43400 Serdang, Malaysia.
[3] Centre for Foundation Studies in Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
[4] Forest Department, PO Box 68, 90009 Sandakan, Sabah, Malaysia.
Received 18th August 2005, accepted in revised form 24th April 2006.

**ABSTRACT** Detailed investigation on the leaves and barks of *Glycosmis chlorosperma* has led to the isolation and identification of a chalcone, dihydroglychalcone **(1)** and three sulphones - dambullin **(2)**, sakambullin **(3)** and methylgerambullin **(4)**, along with the common triterpene, stigmasterol. Compounds **(1)** **(2)** and **(3)** showed cytotoxic activity towards CEM-SS T-lymphoblastic cell lines. However, only dambullin **(2)** showed activity against pathogenic bacteria and fungi. We wish to report the isolation and bioassay results of the crude extracts and pure compounds.

**ABSTRAK** Kajian mendalam pemencilan bahan kimia dari daun dan kulit batang *Glycosmis chlorosperma* telah berjaya mengenali satu calkon, dihidroglicalkon **(1)** dan tiga sulfon - dambullin **(2)**, sakambullin **(3)** dan metilgerambullin **(4)**, bersama dengan triterpena stigmasterol. Sebatian **(1)**, **(2)** dan **(3)** menunjukkan aktiviti sitotoksik terhadap sel T-limfoblastik leukemia CEM-SS. Walau bagaimanapun, hanya dambullin **(2)** sahaja menunjukkan aktiviti terhadap bacteria dan kulat pathogen. Kami ingin melaporkan hasil pemencilan dan biocerakinan ekstrak mentah dan sebatian tulin dari tumbuhan ini.

(*Glycosmis chlorosperma*; Rutaceae; antimicrobial; cytotoxic; antioxidant)

## INTRODUCTION

*Glycosmis chlorosperma* is a small tree or shrub found normally in limestone and lowland forests of tropical South East Asia. Depending on locality, the plant is locally called "cherit murai" or "buluntuh burong" and used traditionally for the treatment of swollen spleen and fever [1]. This genus is known to contain various classes of secondary metabolites such as alkaloids, sulphur-containing amides, coumarins and flavonoids [2, 3, 4, 5]. In our previous work, we have reported the isolation and structural elucidation of dihydroglychalcone **(1)**, dambullin **(2)** and gerambullin, [6]. The plant was recollected from different locations in Sabah, Malaysia, its chemical constituents reexamined and the antimicrobial, cytotoxic and antioxidant activities of the extracts and pure compounds isolated were determined. The results of this bioassay study are described below.

## MATERIAL AND METHODS

### General experimental procedures

Melting points were determined on a Kofler hot stage apparatus and uncorrected. The UV and IR spectra were recorded on a Shimadzu UV-160A and Perkin Elmer FTIR (model 1725X) spectrophotometers, respectively. Mass spectra were recorded by Direct Induction Probe (DIP) using a Shimadzu GCMS-QP5050 spectrometer with ionization induced by electron impact at 70 eV. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a JEOL FTNMR (400 and 100 MHz) spectrometer with tetramethylsilane (TMS) as internal standard. Column chromatography was prepared by using Si gel Merck Kiesegel $PF_{254}$ Art. No. 1.07749.1000 and Merck Kieselgel 60 Art No. 9385.1000. While analytical TLC was performed on commercially available Merck DC-Plasticpolien TLC plastic sheets pre-coated with Kieselgel 60 $F_{254}$, 0.2 mm thickness.

Antibacterial and anifungal tests were evaluated by using the modified paper disc method [9]. The *in vitro* assay was carried out by the MTT method as described by Mosmann [10], using CEM-SS T-lymphoblastic cell lines obtained from the National Cancer Institute, Frederick Maryland USA and cultured in RPMI-1640 medium supplemented with 5% (v/v) fetal calf serum. For the antioxidant activity, DPPH free radical scavenging activity method was employed as described in [7].

**Plant material**

The leaves and stem barks of *Glycosmis chlorosperma* were collected on November 2001 from Tawau, Sabah, Malaysia. Voucher speciments have been deposited in Forest Research Centre, Sepilok, Sandakan, Sabah, Malaysia with accession no. FRCSE 442.

**Extraction and isolation**

The air-dried ground leaves (761.6 g) were extracted sequentially with pet-ether, chloroform and methanol at room temperature. The leaves extracts were filtered and concentrated to give dark viscous semisolids weighing 3.6, 15.5 and 16.8 g, respectively. Similarly, the dried barks gave another three samples of crude extracts weighing 2.5, 3.7 and 10.0 g, respectively. The $CHCl_3$ leaves extract (14.0 g) was separated by flash column chromatography and eluted with pet-ether with gradual increase in $CHCl_3$ and MeOH to give 52 fractions of 100 ml each. Further purification of fractions 22-29 by column chromatography gave yellowish solid which was recrystallized with $CHCl_3$ to give dihydroglychalcone **(1)** as yellowish needles. Similarly, further separation of fractions 35-39 and 44-46 by chromatographic techniques gave dambullin **(2)** and sakambullin **(3)**, respectively. Chromatographic isolation of the MeOH leaves extract gave another sample of dihydroglychalcone **(1)**, dambullin **(2)**, methylgerambullin **(4)** and the common stigmasterol.

**Dihydroglychalcone-A (1):**

$C_{22}H_{24}O_5$, yellow needles, mp 126-128°C. UV $\lambda_{max}$ (MeOH): 243.2, 293.6 and 343.2 nm (chalchone [8]. IR (KBr): $\nu_{max}$ 3431 (OH), 1638 (C=O) $cm^{-1}$. $^1H$ and $^{13}C$ NMR spectral data: see the literature [6]. MS m/z (%): 368 (75), 353 (30), 313 (26), 234 (13), 219 (100), 206 (18), 191 (69), 179 (83), 134 (34), 121 (41) and 91 (28).

**Dambullin (2):**

$C_{17}H_{23}NO_4S$, white needles, mp 145-147°C. UV $\lambda_{max}$ (MeOH): 276.8, 224.2 and 211.6 nm. IR (KBr): $\nu_{max}$1657NC=O), 1304, 1240 ($SO_2$) $cm^{-1}$. $^1H$-NMR (400 MHz, $CDCl_3$): δ 7.36 (*d*, 14.79 Hz, 1H, H-3), 7.09 (*d*, 8.79 Hz, 2H, H-10 and H-14), 6.88 (*d*, 8.79 Hz, 2H, H-11 and H-13), 6.80 (*d*, 14.79 Hz, 1H, H-4), 5.91 (*br t*, 1H, H-6), 5.49 (*t*, 7.19 Hz, 1H, H-2'), 4.50 (*d*, 6.79 Hz, 2H, H-1'), 3.61 (*q*, 6.79, Hz, 2H, H-7), 2.99 (*s*, 3H, H-1), 2.81 (*t*, 6.79 Hz, 2H, H-8), 1.80 (*s*, 3H, H-5') and 1.75 (*s*, 3H, H-4'). $^{13}C$-NMR (100 MHz, $CDCl_3$): δ161.4 (C-5), 157.8 (C-12), 138.9 (C-3), 138.4 (C-3'), 135.4 (C-4), 129.8 (C-9), 129.6 (C-10 and C-14), 119.5 (C-2'), 114.9 (C-11 and C-13), 64.7 (C-1'), 42.5 (C-1), 41.3 (C-7), 34.2 (C-8), 25.8 (C-5') and 18.2 (C-4'). MS m/z (%): 337 (5), 282 (15), 269 (30), 204 (34), 188 (17), 161 (15), 150 (33), 133 (21), 120 (100), 107 (72), 91 (40).

**Sakambullin (3):**

$C_{17}H_{23}NO_5S$, colorless needles, mp 115-117°C. UV $\lambda_{max}$ (MeOH): 278.4, 264.6, 207.6 and 233.0 (sh) nm. IR (KBr): $\nu_{max}$3350 (OH), 1664 (NC=O), 1302 and 1236 ($SO_2$) $cm^{-1}$. $^1H$-NMR (400 MHz, $CDCl_3$): δ 7.36 (*d*, 15.11 Hz, 1H, H-3), 6.81 (*d*, 7.31 Hz, 1H, H-13), 6.78 (*d*, 14.67 Hz, 1H, H-4), 6.76 (*d*, 3.18 Hz, 1H, H-10), 6.63 (*dd*, 7.31 Hz, 3.18 Hz, 1H, H-14), 5.84 (*br* 1H, NH), 5.70 (*br s*, 1H, OH), 5.48 (*t*, 6.87 Hz, 1H, H-2'), 4.56 (*d*, 6.87, 2H, H-1'), 3.60 (*q*, 6.39 Hz, 2H, H-7), 2.99 (*s*, 3H, H-1), 2.77 (*t*, 6.87 Hz, 2H, H-8), 1.80 (*s*, 3H, H-5') and 1.74 (*s*, 3H, H-4'). $^{13}C$-NMR (100 MHz, $CDCl_3$): δ 161.5 (C-5), 146.3 (C-11), 144.9 (C-12), 139.1 (C-3), 138.8 (C-9), 135.5 (C-4), 131.1 (C-3'), 120.2 (C-14), 119.3 (C-2'), 114.7 (C-10), 112.3 (C-13), 65.9 (C-1'), 42.6 (C-1), 41.2 (C-7), 34.6 (C-8), 25.9 (C-4') and 18.3 (C-5'). MS m/z (%): 353 (5), 285 (26), 204 (15), 191 (5), 148 (6), 136 (100), 123 (5), 91 (7), 71 (4).

**Methylgerambullin (4):**

$C_{23}H_{31}NO_5S$, white needles, mp 88-89°C. IR (KBr): $\nu_{max}$1653 (NC=O), 1293 and 1253 ($SO_2$) $cm^{-1}$. $^1H$-NMR (400 MHz, $CDCl_3$): 7.02 (*d*, 8.78 Hz, 1H, H-3), 6.88 (*d*, 8.78 Hz, 1H, H-4), 7.02 (*d*, 8.12 Hz, 2H, H-10 and H-14), 6.85 (*d*, 8.12 Hz, 2H, H-11 and H-13), 5.47 (*t*, 7.55 Hz, 1H, H-2'), 5.10 (*t*, 6.64 Hz, 1H, H-7'), 4.51 (*m*, 2H, H-1'), 3.62 (*m*, 2H, H-7), 3.05 (*s*, 3H, N-$CH_3$), 2.88 (*s*, 3H, H-1), 2.82 (*m*, 2H, H-8), 2.10 (*m*, 4H, H-5', H-6'), 1.73 (*s*), 1.68 (*s*), 1.61 (*s*). $^{13}C$-NMR (100 MHz, $CDCl_3$): δ 1263.1 (C-5), 158.3 (C-12),

130.3 (C-3), 130.0 (C-10), 129.9 (C-4), 124.0 (C-7'), 119.7 (C-2'), 115.5 (C-11), 65.1 (C-1'), 52.5 (C-7), 42.7 (C-1), 39.8 (C-5'), 34.4 (N-$CH_3$), 34.2 (C-8), 26.6 (C-6'), 25.9, 17.9 and 16.8 (C-4', C-9' and C-10').

**Antimicrobial activity**

The five bacteria used were isolated from diseased shrimp culture in ponds from South Sulawesi, Indonesia and grown on Tryptic Soya Agar medium. The fungi used were grown on Potato Dextrose Agar medium. The biological activity of the crude extracts and pure compounds were tested by using the modification of the agar diffusion method [9]. The crude extracts and the pure compounds were dissolved in ethanol and applied on sterile paper discs at 100μg/ml and 10 μg/ml, respectively. The diameter of inhibition was measured after 24 hours of incubation with streptomycin, nystatin and methanol used as control.

**Cytotoxic assay**

The crude extracts and pure compounds were tested for their cytotoxic activity using MTT assay [10] and performed in 96-flat bottom microwell plates. 100 μl of exponentially growing cell suspensions at concentration of $1x10^5$ cell/ml were seeded into the microwell plates in the presence of different concentration of the samples (30.0, 10.0, 3.0, 1.0, 0.3, 0.1, 0.03 μg/ml and control) and incubated for three days. 20 μl of MTT solution was added to each well followed by another 4 hours of incubation at 37°C. The assay of each concentration was performed in triplicate and the cultured plates were kept at 37°C (5% v/v $CO_2$) for four days. Cytotoxicity was determined by using an inverted microscope and the $IC_{50}$ values were calculated.

**Antioxidant assay**

The samples were tested for antioxidant activity by means of DPPH free radical scavenging activity [7]. Stock solutions of the extracts (100 mg) and pure compounds (1 mg) were prepared individually by dissolving them in 1 ml of MeOH. The stock solution was serially diluted in 96-well microplates to varying concentrations, topping from 500 μg/ml down to 7.8 μg/ml. Then 5 μg of methanolic DPPH solution was added to each well, shaken, wrapped in aluminium foil and placed in the dark. After 30 minutes, the optical density of the solution was read from ELISA reader at wavelength 517 nm and percentage inhibition calculated.

## RESULTS AND DISCUSSION

After extensive chromatographic work up, the $CHCl_3$ leaves extract furnished dihydroglychalcone **(1)** as the major component, dambullin **(2)** and sakambullin **(3)**. Compound **(1)** was isolated as white needle-shaped crystal with mp 130-132°C. Dambullin **(2)** was isolated as white needle-shaped crystals with mp 145-147°C and the MS indicated the presence of molecular ion peak at m/z 337 which corresponded to molecular formula $C_{17}H_{23}NO_4S$. Similarly, sakambullin **(3)** was also isolated as white needles with mp 115-117°C but with molecular ion peak 16 mass unit higher at m/z 353 for $C_{17}H_{23}NO_5S$. The $^1$H-NMR spectral data of the two compounds are also similar with the exception at the aromatic region due to the presence of a hydroxyl group at position C-11 in sakambulin. In this compound, the three aromatic protons at H-10, H-13 and H-14 occurred at δ 6.76 (*d*, 3.18 Hz), 6.81 (*d*, 7.31 Hz) and 6.63 (*dd*, 7.31 Hz, 3.18 Hz), respectively. However, the aromatic protons in dambullin occurred as two simple doublets at δ 6.88 (*d*, 8.79 Hz) and 7.09 (*d*, 8.79 Hz) due to equivalent protons H-11/H-13 and H-10/H-14, respectively. Both dambullin **(2)** and sakambullin **(3)** were previously isolated from *Glycosmis angustifolia* [11].

Chromatographic separation work on the MeOH leaves extract led to isolation of another batch of dihydroglychalcone **(1)** and dambullin **(2)** together with methylgerambulin **(4)** and stigmasterol. Methylgerambullin **(4)** was afforded as white needles, mp 88-89°C. The UV spectrum of **(4)** showed the distinct maxima at 260 nm typical of unsaturated tertiary amide and confirmed by the occurrence of a sharp band at 1653 $cm^{-1}$ in the IR spectrum [4]. A pair of aromatic doublets each integrated for two protons in the $^1$HNMR spectrum were observed at δ 7.02 (*d*, 8.12 Hz), 6.85 (*d*, 8.12 Hz) assigned to protons at H-10/H-14 and H-11/H-13, respectively. In both extracts, the major constituent was found to be compound **(1)**. The structures of the various components were established by detailed NMR techniques including DEPT, COSY, NOESY and HMBC spectroscopic methods, direct comparison with previously isolated compounds and literature values [11].

The $CHCl_3$ extract of the leaves showed strong activity towards fungi *Penicillium citrinum,*

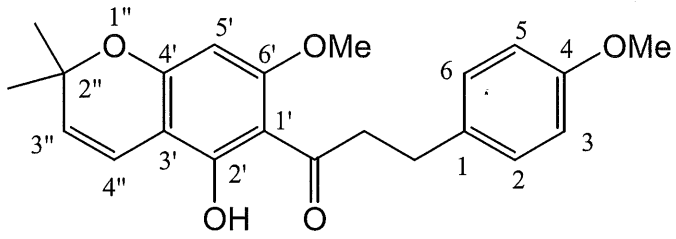
*Penicillium* sp. and *Aspergillus candidus*, with the inhibition diameter > 15.0 mm. This extract also exhibited moderate activity towards fish pathogenic bacteria *Vibrio harveyii, V. costicola, V. alginolyticus* and *Pseudomonas* sp. Of the two pure compounds tested, only dambullin **(2)** gave similar results but sakambullin **(3)** exhibited weak activity. Compound **(2)** showed moderate activity against bacteria (12.0-14.0 mm), and strongly active against fungi with inhibition zones ranges from 17.0 to 21.5 mm in diameter. Hence, the activity shown by the $CHCl_3$ extract is due to the presence of this compound (Table 1) since **(2)** was isolated from this fraction. Except for the MeOH bark extract which gave moderate activity against *Vibrio costicola*, all other extracts were found to be inactive against the microbes tested. The petroleum ether and $CHCl_3$ leaves extracts of the plant have been reported to be active against four species of bacteria, *Bacillus subtilis* (mutant), *Bacillus subtilis* (wild), *Pseudomonas aeruginosa* and *Staphylococcus aeruginosa* [6].

In the cytotoxicity test, the pet ether, $CHCl_3$ and MeOH extracts demonstrated significant inhibition on the growth of CEM-SS T-lymphoblastic cell line with $IC_{50}$ value less than 10.0 μg/ml. Two of the compounds isolated from the $CHCl_3$ extract, dihydroglychalcone **(1)** and sakambullin **(3)**, inhibited the growth of the cancer cells with $IC_{50}$ values of 0.4 and 0.7 μg/ml, respectively, while dambullin (**2**), showed slightly lower $IC_{50}$ value of 2.2 μg/ml. However, the bark extracts showed weak cytotoxic activity. In the antioxidant activity test, all the extracts can be considered as inactive in comparison with the vitamin C and quarcetin standards used. Due to lack of pure samples, antioxidant assay was not carried out and the results of the two bioassays are summarized in Table 2.

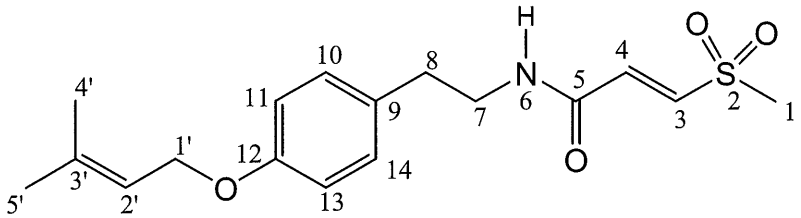
**Acknowledgement** The authors would like to thank the Malaysian Government for financial support under the IRPA programme and Participatory Development of Agricultural Technology Project (PAATP) of the Agency for Agricultural Research and Development (AARD), Government of Indonesia, for scholarship award (Rosmiati).

## REFERENCES

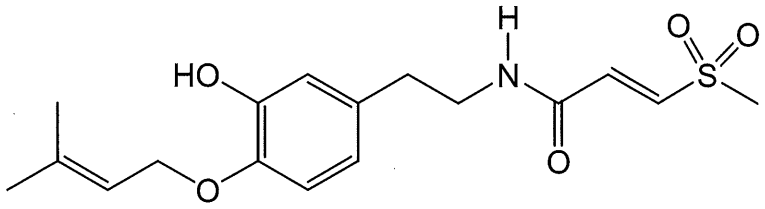
1. Ridley, H.N. (1967). *The Flora of the Malay Peninsular I.* Reeve, London.
2. Greger, H., Zechner, G., Hofer, O. and Vajrodaya S. (1996). Bioactive amides from *Glycosmis* species. *J Nat Prod* **59**: 1163.
3. Ito, C., Kondo, Y., Ruangrungsi, N. and Furukawa, H. (1999). New quinazoline alkaloids from *Glycosmis cochinensis*. *Chem. Pharm. Bull.* **47**: 1491-1493.
4. Hofer, O., Greger, H., Lukaseder, B., Vajrodaya, S. and Bacher, M. (2000). Prenylated sulfonyl amides from *Glycosmis* species. *Phytochemistry* **54**: 207-213.
5. Wu, T.S., Chang, F.C. and Wu, P.L. (1995). Flavanoids, amidosulfides and an alkaloid from leaves of *Glycosmis citrifolia*. *Phytochemistry* **39**: 1453.
6. Rahmani, M., Leng, K.W., Ismail, H.B.M., Taufiq-Yap, Y.H., Sukari, M.A., Ali, A.M. and Kulip, J. (2004). A new flavonoid and sulphur-containing amides from *Glycosmis chlorosperma*. *Nat. Prod. Res.* **18**: 85-88.
7. Lee, S.K., Zakaria, H.M., Chung, H.S., Luyengi, L., Gamez, E.J.C., Metha, R., Kinghorn, A.D. and Pezzuto, J.M. (1998). Evaluation of the antioxidant potential of natural products. *Combinat. Chem. High Throughput Screen* **1**: 35-46.
8. Mabry, T.J., Markham, K.R. and Thomas, M.B. (1970). *The Systematic Identification of Flavonoids*. Springer-Verlag, Heidelberg.
9. Cowan, S.T. (1985). *Manual for the Identification of Medical Bacteria*. Cambridge University Press, Cambridge.
10. Mosmann, T.R. (1983). Rapid colorimetric for cellular growth and survival: Application to proliferation and cytotoxic assays. *J. Immonol. Methods* **65**: 55-58.
11. Greger, H., Hofer, O., Zechner, G., Hadacek, F. and Wurz, G. (1994). Sulphones derived from methylthiopropenoic acid amides from *Glycosmis angustifolia*. *Phytochemistry* **37**: 1305-1310.



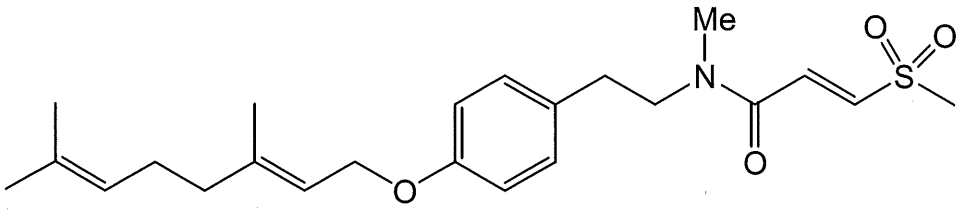

**(1)**




**(2)**




**(3)**




**(4)**

**Figure 1.** Extracts from *Glycosmis chlorosperma*

**Table 1.** Antimicrobial test of the compounds, crude extracts of the leaves and stem barks of *Glycosmis chlorosperma*

| SAMPLES | CONC. (µg/ml) | INHIBITION DIAMETER OF MICROBES TESTED (IN mm)[#] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J |
| Leaves | | | | | | | | | | | |
| Pet. Ether | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $CHCl_3$ | 100 | 8.0 | 7.0 | 9.5 | 0 | 10.0 | 22.4 | 17.5 | 0 | 19.2 | 0 |
| MeOH | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stem barks | | | | | | | | | | | |
| Pet. ether | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $CHCl_3$ | 100 | 13.0 | 12.4 | 11.0 | 0 | 10.6 | 0 | 0 | 0 | 0 | 0 |
| MeOH | 100 | 0 | 11.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Compounds | | | | | | | | | | | |
| Dambullin (**2**) | 10 | 13.5 | 12.0 | 0 | 14.0 | 14.0 | 21.5 | 18.0 | 0 | 17.0 | 0 |
| Sakambullin (**3**) | 10 | 6.5 | 0 | 0 | 0 | 0 | 8.3 | 7.5 | 0 | 0 | 0 |
| Standards | | | | | | | | | | | |
| Streptomicin | 10 | 12.0 | 22.0 | 19.5 | 0 | 17.0 | - | - | - | - | - |
| Nystatin | 10 | - | - | - | - | - | 0 | 0 | 0 | 0 | 0 |

[#]A: *Vibrio harveyii*, B: *V.costicola*, C: *V.alginolyticus*, D: *V.mimicus*, E: *Pseudomonas sp*: F: *Penicillin citrinum*, G: *Penicillin sp*, H: *Aspergillus niger*, I: *Aspergillus candidus*, J: *Aspergillus clavatus*, (-): Not tested.

**Table 2.** Cytotoxic and antioxidative activities of crude extracts and isolated compounds from *Glycosmis chlorosperma*

| EXTRACTS | CYTOTOXIC ACTIVITY $IC_{50}$ VALUES (μg/ml) | ANTIOXIDANT ACTIVITY $IC_{50}$ VALUES (μg/ml) |
|---|---|---|
| Leaves | | |
| Pet. ether | 8.7 | 229.2 |
| $CHCl_3$ | 0.7 | 229.2 |
| MeOH | 0.5 | 229.2 |
| Barks | | |
| Pet. ether | 29.4 | 200.0 |
| $CHCl_3$ | 19.8 | 200.0 |
| MeOH | 18.3 | 187.5 |
| Compounds | | |
| Dihydroglychalcone-A (**1**) | 0.7 | - |
| Dambullin (**2**) | 2.2 | - |
| Sakambullin (**3**) | 0.4 | - |
| Standards | | |
| Doxorubicin | ≤0.1 | - |
| Vitamin A | - | 14.3 |
| Quarcetin | - | 78.5 |