Antimicrobial, anticancer, and antioxidant compounds from *Premna resinosa* growing in Saudi Arabia

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ABSTRACT
Context: *Premna resinosa* (Hochst.) Schauer (Lamiaceae) is used in many places to treat bronchitis, respiratory illness and convulsions of the rib cage.

Objective: This study evaluates the anticancer, antimicrobial and antioxidant activities of *P. resinosa*, and isolates some responsible constituents.

Materials and methods: The methanol extract of *P. resinosa* aerial parts and its fractions (n-hexane, dichloromethane, ethyl acetate and n-butanol) were tested. Antimicrobial activity was tested using microdilution method against three Gram-positive and four Gram-negative bacteria. The tested concentrations ranged from 4000 to 7.8 µg/mL and MIC values were determined after 24 h incubation. Anticancer activity was evaluated against three human cancer cell lines (Daoy, HepG2 and SK-MEL28) using MTT assay. Antioxidant activity was investigated by DPPH scavenging method and β-carotene-linoleic acid assay.

Results: The greatest antimicrobial activity was exhibited by n-hexane fraction (MIC 10 µg/mL) against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Shigella flexneri*. The n-hexane fraction induced the greatest cytotoxic activity against Daoy, HepG2, and SK-MEL28 cell lines with IC50 values of 9.0, 8.5 and 13.2, respectively. Moreover, the dichloromethane and ethyl acetate fractions showed the highest antioxidant potential. A bioassay-guided fractionation led to the isolation and characterization of seven compounds for the first time, namely, quercetin, 3-methoxy quercetin, kaempferol, 3-methoxy kaempferol, myricetin, 3,7,3'-trimethyl ether, lupeol, and stigmasterol.

Conclusion: Our results indicate that *P. resinosa* is a source for antimicrobial and cytotoxic compounds. However, further work is required to isolate other active principles and to determine the mechanism of action.

Introduction
The genus *Premna* L. (Verbenaceae), with 200 species worldwide, is distributed chiefly in tropical and subtropical Asia, Africa, Australia, and the Pacific Islands (Thirumalai et al. 2011). Some *Premna* species are considered as natural resources for antimicrobial, antioxidant, antipyretic, hypoglycaemic, cardiotonic, and diuretic agents (Hymavathi et al. 2009). *Premna resinosa* (Hochst.) Schauer grows as a shrub, small bushy tree, or subscandent and the inflorescence is few-flowered terminal with very short side shoots (Verdcourt 1992). In Indian folk medicine, *P. resinosa* leaves were used as laxative agent (Patel et al. 2013) and to treat bronchitis. In addition to the above the stem paste of *P. resinosa* was used to cure swellings and body pains (Joshi et al. 2013). In Kenya it is called ‘small rib plant or mukarakara’ and it is used to treat convulsions of the rib cage (Pakia, 2006) and for the management of respiratory illness (Njeru et al. 2015). To date, available present pharmacognostical, pharmacological and phytochemical studies on aerial part of *P. resinosa* are very infrequent. On the basis of previous reports on pharmacological activities of *Premna* species and in continuation of our ongoing research for bioactive compounds from interesting medicinal plants, *P. resinosa* was selected in this study. The present study evaluates the antimicrobial, anticancer and antioxidative effects of the aerial part of *P. resinosa*. In addition, in this investigation we report the isolation and characterization of active constituents by way of the activity-guided fractionation procedure.

Materials and methods
General
UV-spectra were determined using a spectrophotometer (Beckman DU 7500; Beckman-Coulter, Fullerton, CA). The mass spectral analyses were obtained on a microTOF high-resolution mass spectrometer (Bruker Daltonik, Bremen, Germany).
The nuclear magnetic resonance analyses (1H, 13C-NMR and 2D-NMR spectra) were recorded on a Bruker AMX-500 spectrometer in deuterated methanol, dimethyl sulfoxide and chloroform. Column chromatography was conducted using silica gel 60 (Kieselgel 60, 0.062-0.2 mm, Merck, Darmstadt, Germany) and LiChroprep RP-18 (2540 lm, Merck). All TLC experiments were conducted on pre-coated Merck Kieselgel 60 F254 plates and RP-18 F254 (Merck, Darmstadt, Germany).

Plant materials
The Premna resinosa plant was collected from Wadi bin Sayeed located in Riyadh region, Saudi Arabia in January 2011 and identified by Dr. Mohamed Yusuf at the Pharmacognosy Department, College of Pharmacy, King Saud University (KSU). A voucher specimen (15638) was deposited at the Pharmacognosy Department, College of Pharmacy, KSU.

Extraction, fractionation and isolation
Air-dried and powdered P. resinosa aerial parts (1 kg) were exhaustively extracted with methanol using a Soxhlet apparatus (Witeg, Germany) for 4h. The obtained extract was concentrated under reduced pressure using rotary evaporator (Buchi, Flawil, Switzerland) to give a crude methanol extract (90 g). After that, the dried methanol extract was suspended in distilled water and successively partitioned with n-hexane, dichloromethane, ethyl acetate and n-butanol to yield

\[ \text{n-hexane fraction and terpenoids and flavonoids in dichloromethane and ethyl acetate fractions. Because the dichloromethane and ethyl acetate fractions showed similar spectrum on the TLC plate, both fractions were combined together. Consequently, part of the (DCM/EtOAc) combined fraction (3.5 g) was subject to silica gel column chromatography (3 x 40 cm) using CHCl3-MeOH gradient as a mobile phase. According to the TLC results, similar fractions were pooled together to yield seven fractions (F1-F7). Fraction F2 was purified using chromatotron (Harrison Research Laboratories, Palo Alto, CA) with chloroform and methanol gradient as a mobile phase. Only one major compound (5) (myricetin 3,7,3′-trimethyl ether, 10 mg) was obtained from this fraction as yellowish crystals after recrystallization. Fractions F3, F4 and F5 were subjected to column chromatography using sephadex LH-20 as a stationary phase (1.5 x 60 cm) and methanol and water gradient as mobile phase. Two major compounds; compound 3 (kaempferol, 8 mg) and compound 4 (3-methoxy kaempferol 15 mg) were obtained from fraction F3 while only one compound; compound 2 (3-methoxy quercetin, 36 mg) was yielded from fraction F4. Fraction F5 yielded a major compound; compound 1 (quercetin, 25 mg).

The n-hexane fraction (3 g) was subjected to silica gel column chromatography (3 x 40 cm) using n-hexane and ethyl acetate in a gradient mode of analysis to give 10 fractions. According to the results of antimicrobial, cytotoxic and antioxidant tests, the fractions of n-hexane extract were subjected for further fractionation and purification. Fractions F1 and F2 were subjected to column chromatography (1.5 x 60 cm), to obtain their main compounds; compound 6 (lupeol, 46 mg) and compound 7 (stigmasterol, 41 mg).

Quercetin (compound 1)
Yellowish crystalline solid; ESIMS m/z 302 [M+], 301 [M-H]. 1H NMR (DMSO-d6, 500 MHz) δ: 7.69 (1H, d, J = 2.0 Hz, H-2′), 7.55 (1H, dd, J = 2.0/8.5 Hz, H-6′), 6.89 (1H, d, J = 8.5 Hz, H-5′), 6.42 (1H, d, J = 1.5 Hz, H-8), 6.19 (1H, d, J = 1.5 Hz, H-6); 13C NMR (DMSO-d6, 500 MHz) δ: 175.8 (s, C-4), 163.9 (s, C-7), 160.7 (s, C-5), 146.8 (s, C-2), 156.1 (s, C-9), 147.7 (s, C-4′), 145.0 (s, C-3′), 135.7 (s, C-3), 119.9 (d, C-6′), 121.9 (s, C-1′), 115.0 (d, C-2′), 115.6 (d, C-5′), 102.9 (s, C-10), 98.2 (d, C-6), 93.3 (d, C-8) (Figure 1).

Methoxy quercetin (compound 2)
Yellowish needle crystal; ESIMS m/z 315 [M-H], 300 [M-H-CH3]. 1H NMR (DMSO-d6, 500 MHz) δ: 7.55 (1H, d, J = 2.5 Hz, H-2′), 7.49 (1H, dd, J = 2.0/-8.5 Hz, H-6′), 6.91 (1H, d, J = 8.5 Hz, H-5′), 6.43 (1H, d, J = 1.5 Hz, H-8), 6.12 (1H, d, J = 1.5 Hz, H-6); 13C NMR (DMSO-d6, 500 MHz) δ: 177.9 (s, C-4), 164.0 (s, C-7), 161.3 (s, C-5), 155.6 (s, C-2), 156.3 (s, C-9), 148.7 (s, C-4′), 145.2 (s, C-3′), 137.6 (s, C-3), 120.5 (d, C-6′), 120.7 (s, C-1′), 115.4 (d, C-2′), 116.0 (d, C-5′), 104.1 (s, C-10), 98.7 (d, C-6), 93.6 (d, C-8), 59.7(C-3, OCH3) (Figure 1).

Kaempferol (compound 3)
Yellowish powder; ESIMS m/z 286 [M+]. 1H NMR (CD3OD, 500 MHz) δ: 8.12 (2H, d, J = 8.5 Hz, H-2′), 8.12 (2H, d, J = 8.5 Hz, H-6′), 6.94 (2H, d, J = 8.5 Hz, H-5′), 6.43 (1H, d, J = 2.1 Hz, H-8), 6.21 (1H, d, J = 2.1 Hz, H-6); 13C NMR (CD3OD, 500 MHz) δ: 177.4 (s, C-4), 162.6 (s, C-7), 160.6 (s, C-5), 158.3 (s, C-9), 158.3 (s, C-4′), 148.1 (s, C-2), 130.7 (s, C-3), 130.7 (d, C-2′), 130.7 (d, C-6′), 123.8 (s, C-1′), 116.4 (d, C-3′), 116.4 (d, C-5′), 104.6 (s, C-10), 99.3 (d, C-6), 94.5 (d, C-8) (Figure 1).

Methoxy kaempferol (compound 4)
Yellowish solid; ESIMS m/z 300 [M+]. 1H NMR (DMSO-d6, 500 MHz) δ: 7.94 (2H, d, J = 8.5 Hz, H-2′), 7.94 (2H, d, J = 8.5 Hz, H-6′), 6.95 (2H, d, J = 9 Hz, H-5′), 6.44 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, d, J = 2.0 Hz, H-6); 13C NMR (DMSO-d6, 500 MHz) δ: 177.9 (s, C-4), 164.2 (s, C-7), 161.3 (s, C-5), 158.3 (s, C-4′), 156.3 (s, C-9), 155.6 (s, C-2), 137.6 (s, C-3), 130.1 (d, C-2′), 130.1 (d, C-6′), 120.5 (s, C-1′), 115.6 (d, C-3′), 115.6 (d, C-5′), 104.1 (s, C-10), 98.7 (d, C-6), 93.7 (d, C-8), 59.7(C-3, OCH3) (Figure 1).

Myricetin 3,7,3′-trimethyl ether (compound 5)
Yellowish solid; ESIMS m/z 360 [M+]. 1H NMR (DMSO-d6, 500 MHz) δ: 7.27 (1H, d, J = 2.5 Hz, H-8), 7.33 (1H, d, J = 2.0 Hz, H-6′), 6.75 (1H, d, J = 2.5 Hz, H-8), 6.37 (1H, d, J = 2.5 Hz, H-6); 13C NMR (DMSO-d6, 500 MHz) δ: 178.0 (s, C-4′), 165.1 (s, C-7), 160.9 (s, C-5), 156.2 (s, C-9), 156.2 (s, C-2), 138.1 (s, C-4′), 137.9 (s, C-3), 104.1 (d, C-2′), 109.7 (d, C-6′), 119.5 (s, C-1′), 148.1 (s, C-3′), 145.6 (s, C-5′), 105.1 (s, C-10), 103.7 (d, C-6), 103.7 (d, C-8), 103.7 (d, C-10)
Determination of the antimicrobial activity

Tested microorganisms

The following microorganisms were used as test organisms in the antimicrobial activity screening: Staphylococcus aureus (ATCC 25923) and (ATCC 29312), Bacillus subtilis (NCTC 10400), Acinetobacter baumannii (BM 4436), Escherichia coli 469-3 (O21: H-), Salmonella typhimurium (ATCC 14028), Shigella flexneri (ATCC- 12022) and Enterococcus faecalis (ATCC 29212).

Broth micro-dilution assay for minimum inhibitory concentrations (MICs)

The broth micro-dilution method described by Mann and Markham (1998) was used with some modifications to determine the MICs values of the crude extract and its fractions as well as the isolated compounds. Utilizing sterile round-bottom 96-well plates, duplicate 2-fold serial dilutions of fraction (100 µL/well) were prepared in the appropriate broth containing 5% (v/v) DMSO to produce a concentration range of 4000–7.8 µg of extract/mL. A bacterial cell suspension (prepared in the appropriate broth) of 100 µL, corresponding to 1/10⁶ CFU/mL, was added in all wells except those in column 10, 11 and 12, which served as saline, extract and media sterility controls, respectively. Controls for bacterial growth without plant extract were also included on each plate. The final concentration of bacteria in the assay was 5 x 10⁵ CFU/mL. Plates were then incubated at 37 °C for 18 h overnight. After incubation, the MIC of each extract was determined as the lowest concentration at which no growth was observed in the duplicate wells. A p-iodonitro-tetra-zolium violet solution (20 µL 0.04%, w/v) (Sigma, Aldrich, St Louis, MO) was then added to the wells. The plates were incubated for a further 30 min, and estimated visually for any change in colour from

Figure 1. Chemical structures of the isolated flavonoids.
yellow to pink indicating reduction of the dye due to bacterial growth. The highest dilution (lowest concentration) that remained yellow corresponded to the MIC. Experiments were performed in duplicate.

**In vitro antiproliferative assay on human cancer cell lines**

**Chemicals and supplies**

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was purchased from Sigma Aldrich (St. Louis, MO). DMEM/high glucose, FBS and penicillin/streptomycin were obtained from Hyclone Laboratories (Logan, UT).

**Cell lines**

Three tumor cell lines were utilized in this study, namely, human medulloblastoma (Daoy), human hepatocellular carcinoma (HepG2) and human melanoma (SK-Mel-28). HepG2 cells were cultured in DMEM/high glucose supplemented with 10% FBS, 2 mM l-glutamine and 1% penicillin/streptomycin. Daoy and SK-Mel-28 were cultured in DMEM/F12 supplemented with 10% FBS, 2 mM l-glutamine and 1% penicillin/streptomycin.

**Screening of antiproliferative activity by MTT assay**

The crude extract and its fractions as well as the isolated compounds were evaluated at the Cell Culture Laboratory, College of Pharmacy, King Saud University, in a primary three cell line-one concentration (25 μg/mL) anticancer assay against the previously mentioned cell lines. The cytotoxic effect was evaluated by testing the capacity of the reducing enzymes present in viable cells to convert MTT to formazan crystals (Al-Salahi et al. 2014). Briefly, cells cultured in complete medium were seeded into 96-well microtiter plates (in quintuplicates) with 2 × 10^4 cells per well and incubated at 37 °C under a humidified atmosphere of 5% CO₂ for 24 h. The cell medium in test wells were then changed to serum free medium (SFM) containing 25 μg/mL of the tested crude extract, fractions and isolated compounds, while the cell medium in control wells were changed to SFM containing an equivalent volume of solvent (dimethyl sulfoxide 'DMSO'). After incubation at 37 °C for 24 h, SFM in control and test wells were replaced by 100 μL/well of MTT; 0.5 mg/mL in phosphate-buffered saline (PBS) and incubated at 37 °C for an additional 3 h. MTT solution was removed and the purple formazan crystals formed at the bottom of the wells were dissolved using isopropanol (100 μL/well) with shaking for 1 h at room temperature. The absorbance at 549 nm was read on a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT). All the measurements were performed in quintuplicates. The dose response curves of the compounds effecting ≥50% inhibition in one-dose prescreening for each cell line were established utilizing concentrations of 25, 12.5, 6.25, 3.125, 1.56 and 0.78 μg/mL, and the concentrations causing 50% cell growth inhibition (IC₅₀) were calculated. Dasatinib, a potent inhibitor of BCR-ABL and SRC family kinases (Lombardo et al. 2004), was used as a standard for comparative purposes.

**Studies of antioxidant activity**

**Scavenging activity of DPPH radical**

The 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity. The method was carried out as described by Brand et al. (1995) to measure the free radical scavenging capacity of the crude extract, fractions and isolated compounds. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour, typical for free DPPH radical decays, and the change in absorbency at λ = 517 nm is followed spectrophotometrically. The methanol crude extracts and its fractions were re-dissolved in methanol and 5% ethanol, respectively, and various concentrations (10, 50, 100, 500 and 1000 μg/mL) of each were used. The assay mixture contained in a total volume of 1 mL, 500 μL of the extract, 125 μL prepared DPPH (1 mM in methanol) and 375 μL solvent (methanol or 5% ethanol). After 30 min incubation at 25 °C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation:

\[
\text{% radical scavenging activity} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100
\]

**β-Carotene-linoleic acid assay**

The antioxidant activity of the crude extract, fractions and isolated compounds was evaluated, using the β-carotene bleaching method described by Velioglu et al. (1998) and Mohd-Esa et al. (2010) with some modifications. A 0.2 mg/mL β-carotene solution (1 mL) in chloroform was added to flasks containing 0.02 mL of linoleic acid and 0.2 mL of Tween-20. The chloroform was removed at 40 °C using a rotary evaporator. The resultant mixture was immediately diluted with 100 mL of distilled water and mixed for 1-2 min to form an emulsion. A mixture prepared similarly but without β-carotene, was used as a blank. A control containing 0.2 mL of 80% (v/v) methanol instead of extract was also prepared. A 5 mL aliquot of the emulsion was added to a tube containing 0.2 mL of the sample extract (1 mg/mL). Rutin (1 mg/mL) was used as a standard. The tubes were placed in a water bath at 40 °C for 2 h. Absorbance was read at 470 nm at 15 min intervals, using a UV–visible spectrophotometer (UV mini-1240, Shimadzu, Japan). The antioxidant activity was calculated using the equation:

\[
\text{% of antioxidant activity} = \left[ 1 - \left( \frac{A_0 - A_t}{A_0^* - A_t^*} \right) \right] \times 100
\]

where \(A_0\) and \(A_0^*\) are the absorbance values measured at zero time of incubation for sample extract and control, respectively. \(A_t\) and \(A_t^*\) are the absorbance values for sample extract and control, respectively, at \(t = 120\) min.

**Statistical analysis**

All data are presented as mean ± standard deviation (SD) for experiments performed in triplicate. Statistical analysis of the data was performed by analysis of variance (ANOVA, IBM, SPSS, version 22). Significance difference was indicated by probability values of \(p \leq 0.05\).

**Results**

In the course of our screening for antimicrobial, cytotoxic and antioxidant activities, the crude extract of the aerial part of *Prema resinos*, fractions and isolated compounds were evaluated.

**Antimicrobial activity**

A preliminary screening using agar diffusion assay for the crude methanol extract and its fractions showed a strong antimicrobial
activity against almost bacterial strains (data not shown). Generally, the dichloromethane fraction exhibited the highest antimicrobial activity. Consequently, the MIC-values were determined only for the active fractions. MIC values are demonstrated in Table 1, which showed that the Gram-positive bacterial strains were more susceptible to *P. resinosa* fractions than the Gram-negative bacterial strains. The *n*-hexane, dichloromethane and ethyl acetate fractions showed a strong antimicrobial activity against two *Staphylococcus aureus* strains, *Bacillus subtilis*, *Enterococcus faecalis*, *A. baumannii*, *E. coli*, *S. typhimurium* and *S. flexneri* with MIC values ranging between 0.01 and 0.25 mg/mL (Table 1).

### Table 1. Minimum inhibitory concentrations (mg/mL) of the *P. resinosa* crude methanol extract and its fractions.

| Organism tested     | Crude methanol Ex. | *n*-Hexane Fr. | Dichloromethane Fr. | EtOAc. Fr. | *n*-Butanol Fr. |
|---------------------|--------------------|----------------|---------------------|------------|-----------------|
| *S. aureus*<sup>a</sup> | 0.06              | 0.01           | 0.25                | 0.06       | 1               |
| *S. aureus*<sup>b</sup> | 0.25              | 0.12           | 0.25                | 0.5        | 0               |
| *B. subtilis*       | 0.25              | 0.06           | 0.25                | 0.12       | 1               |
| *E. faecalis*       | 0.03              | 0.01           | 0.01                | 0.03       | 0.5             |
| *A. baumannii*      | 1.00              | 0.25           | 0.25                | 0.5        | 1               |
| *E. coli*           | 0.12              | 0.03           | 0.25                | 0.06       | 1               |
| *S. typhimurium*    | 1.00              | 0.5            | 1                   | 1          | 2               |
| *S. flexneri*       | 0.12              | 0.01           | 0.01                | 0.03       | 0.12            |

<sup>a</sup>*Staphylococcus aureus* ATCC 29312, <sup>b</sup>*Staphylococcus aureus* ATCC-25923, *Bacillus subtilis* NCTC 10400, *Acinetobacter baumannii* BM 4436, *Escherichia coli* 469-3 (O21: H<sup>+</sup>), *Salmonella typhimurium* ATCC 14028, *Shigella flexneri* ATCC 12022.

Cytotoxic activity

As shown in Table 2, the crude methanol extract and its *n*-hexane and dichloromethane/ethyl acetate combined fractions showed the strongest cytotoxic activity at both concentrations tested (25 and 50 μg/mL). Daoy cell line showed more sensitivity to the extract and fractions than the HepG2 cell line. The most interesting results against Daoy cell line were observed with the crude extract, *n*-hexane and combined dichloromethane/ethyl acetate fractions with IC<sub>50</sub> values of 5.2, 9.0 and 17.8 μg/mL, respectively (Table 2). Four compounds isolated from dichloromethane/ethyl acetate fraction namely quercetin, 3-methoxy quercetin, kaempferol and myricetin 3,7,3′-trimethyl ether showed interesting cytotoxic activity with IC<sub>50</sub> values of 10.5, 19.5, 21.1 and 7.0 μg/mL, respectively (Table 2).

Antioxidant activity

The results of the antioxidant activity are presented in Table 3. In the β-carotene-linoleic acid model system, the dichloromethane fraction was able to inhibit the discoloration of β-carotene at a concentration of 1000 μg/mL with total antioxidant value of 90.6% followed by ethyl acetate fraction (86.1%), as shown in Table 3. Also, compound 1 (quercetin) was able to inhibit the discoloration of β-carotene with total antioxidant value of 94.4% (Table 3). The lowest antioxidant activity was revealed by myricetin 3,7,3′-trimethyl ether (80.9%). In addition, results of the DPPH radical scavenging method demonstrated that also the dichloromethane fraction exhibited the highest free radical scavenging activity almost similar to the effect of ascorbic acid (Table 3). It showed at 100, 500 and 1000 μg/mL a free radical resistance.

![Table 1. Minimum inhibitory concentrations (mg/mL) of the P. resinosa crude methanol extract and its fractions.](image-url)
scavenging activity of 90.8, 97.1 and 96.5%, respectively. Moreover, as demonstrated in Table 3, quercetin exhibited the highest antioxidant and free radical scavenging activity at 1000 µg/mL (95.8%) among the isolated compounds. 3-Methoxy quercetin and kaempferol showed comparable activities in both assays.

**Discussion**

Our biological study on the aerial part of *P. resinosa* exhibited remarkable antioxidant activity as well as relevant antimicrobial and cytotoxic activities. It is important to point out that this work represents the first report on the antimicrobial, cytotoxic and antioxidant activities of the aerial part of *P. resinosa*. Additionally, from the chemical point of view, this *Premna* species has not been previously investigated and no reports on the isolation of secondary metabolites were found.

A bioassay-guided fractionation of the dichloromethane/ethyl acetate fraction led to the isolation of 5 flavonoids, namely quercetin, 3-methoxy quercetin, kaempferol, 3-methoxy kaempferol and myricetin 3,7,3’-trimethyl ether. In addition, lupeol and stigmasterol were isolated from the *n*-hexane fraction. All compounds were isolated for the first time from the *P. resinosa*.

The antimicrobial activity results of the aerial part of *P. resinosa* crude extract and its fractions were in agreement with data reported recently on the roots of *P. resinosa* which indicated high selective antibacterial, antibacterial and antifungal activities for the roots of *P. resinosa* (Njeru et al. 2015). The results were also in agreement with the antimicrobial activity of *P. integrifolia* which showed a great antimicrobial activity against *Sarcina lutea*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas sp.*, *Klebsiella pneumonia* and *Xanthomonas campestris* (Rahman et al. 2011). Furthermore, the demonstrated antimicrobial activity of quercetin is in agreement with prior data showing that quercetin showed a great antimicrobial activity against *B. cereus* and *S. enteritidis* with MICs values of 0.35 and 0.25 mg/mL respectively (Arima & Danno 2002). Moreover, 3-methoxy quercetin isolated from *Inula viscosa* (L.) Aiton (Asteraceae) showed antimicrobial activity against *B. cereus* and *S. typhimurium* with MIC value of 0.125 mg/mL (Talib et al. 2012).

The interesting cytotoxic activity of *P. resinosa* crude extract and its fractions were in agreement with the data reported on several *Premna* species e.g. *P. herbacea* Roxb., *P. serratifolia* and *P. tomentosa* Willd. (*Selvam et al. 2012; Dhamija et al. 2013; Naidu et al. 2014*). For instance, it was revealed that the ethanol extract of *P. herbacea* and its ethyl acetate fraction inhibited the proliferation of human breast adenocarcinoma cell line (MCF-7) in vitro with IC50 values of 75.51 and 84.04 µg/mL, respectively (Dhamija et al. 2013). It is noteworthy to mention that several

### Table 2. Cytotoxic activity of methanol extract of *P. resinosa*, its fractions and isolated compounds.

| Tested samples | Daoy % Inhibition (µg/mL) | HepG2 % Inhibition (µg/mL) | SK-MEL28 % Inhibition (µg/mL) |
|----------------|---------------------------|---------------------------|--------------------------------|
| Crude methanol ext. | 62.1 | 5.2 ± 2.8 | 40.1 | NT | 0 | NT |
| *n*-Hexane fr. | 64.5 | 9.0 ± 1.9 | 83.9 | 8.5 ± 2.2 | 70.4 | 13.2 ± 4.1 |
| Dichloromethane/ethyl acetate fr. | 62.9 | 17.8 ± 3.5 | 81.4 | 9.8 ± 3.1 | 38.0 | NT |
| *n*-Butanol fr. | 0 | – | 0 | NT | 1.48 | NT |
| Isolated compounds | | | | | | |
| Quercetin | 62.40 | 10.5 ± 2.4 | 23.31 | >95<sup>a</sup> | 0 | >95<sup>a</sup> |
| 3-Methoxy quercetin | 51.07 | 19.6 ± 4.3 | 30.21 | >95<sup>a</sup> | 44 | >95<sup>a</sup> |
| Kaempferol | 54.84 | 21.1 ± 3.1 | 36.56 | >95<sup>a</sup> | 4.86 | >95<sup>a</sup> |
| 3-Methoxy kaempferol | 14.94 | >95 | 33.25 | 25<sup>a</sup> | 65.76 | 18.8 ± 3.8 |
| Myricetin 3,7,3’-trimethyl ether | 65.0 | 7.0 ± 1.2 | 40.72 | >95<sup>a</sup> | 22.15 | >95<sup>a</sup> |
| Lupeol | 36.89 | >95<sup>a</sup> | 0 | >95<sup>a</sup> | 0.045 | >95<sup>a</sup> |
| Stigmasterol | 45 | >95<sup>a</sup> | 39.77 | >95<sup>a</sup> | 37.05 | >95<sup>a</sup> |
| Dasatinib | 76.60 | 7.3 ± 1.6 | 72.03 | 8.2 ± 2.1 | 51.95 | 23.8 ± 4.6 |

**Note:** Percent inhibition of cell survival at a concentration of 25 (µg/mL), relative to control (DMSO-treated cells), Daoy: Medulloblastoma cell line, HepG2: hepatocellular carcinoma cell line, SK-MEL28: melanoma cell line, NT: Not tested. In the column of IC50, means ± SD with same letter notification are not significantly different at (p < 0.05) (n = 3). *Not included in statistical analysis.

### Table 3. Antioxidant and free radical scavenging activity of the fractions and isolated compounds of *P. resinosa*.

| Fractions and isolated compounds | Free radical scavenging activity % (DPPH) assay |
|---------------------------------|-----------------------------------------------|
|                                | Concentration (µg/mL) |
|                                | 10   | 50   | 100  | 500  | 1000 |
| **Total antioxidant activity**  |       |       |       |      |      |
| in % (1000 µg/mL)               |       |       |       |      |      |
| **Fractions**                   |       |       |       |      |      |
| *n*-Hexane                      | 61.4 ± 6.1 | 2.3 ± 1.2 | 9.2 ± 2.3 | 37.1 ± 3.9 | 67.6 ± 3.2d | 77.5 ± 2.7 |
| Dichloromethane                 | 90.6 ± 5.6a | 25.5 ± 4.3 | 69 ± 3.0 | 90.8 ± 4.2a | 97.1 ± 2.1a | 96.5 ± 1.9 |
| Ethyl acetate                   | 86.1 ± 4.5b | 18.1 ± 2.0a | 48.3 ± 5.5 | 81.9 ± 3.1c | 92.5 ± 2.6a | 92.9 ± 2.3a |
| *n*-Butanol                     | 72.0 ± 5.2 | 12.5 ± 2.3 | 21.4 ± 4.7 | 48.8 ± 5.0 | 72.2 ± 3.8d | 85.7 ± 4.1 |
| **Isolated compounds**          |       |       |       |      |      |
| Quercetin                       | 94.4 ± 3.8 | 29.2 ± 4.1 | 81.1 ± 2.8 | 91.2 ± 1.9a | 91.5 ± 1.6ab | 95.8 ± 2.3b |
| 3-Methoxy quercetin             | 90.5 ± 4.3a | 16 ± 3.2 | 55.1 ± 5.1 | 86.1 ± 3.4b | 91.0 ± 2.1a | 92.1 ± 1.9bc |
| Kaempferol                      | 91.2 ± 4.1a | 22.4 ± 5.1 | 74.8 ± 3.2 | 82.3 ± 3.0c | 90.0 ± 2.5a | 92.9 ± 1.6d |
| 3-Methoxy kaempferol            | 86.2 ± 3.9b | 18.8 ± 4.0a | 49.6 ± 4.6 | 82.8 ± 5.1c | 90.5 ± 3.3a | 91.0 ± 2.8cd |
| Myricetin 3,7,3’-trimethyl ether | 80.9 ± 5.3 | 15.4 ± 3.3 | 41.6 ± 2.8 | 63.5 ± 3.2 | 78.1 ± 4.1bc | 87.1 ± 3.0 |
| Rutin                           | 93.1 ± 3.0 | NT | NT | NT | NT | NT |
| Ascorbic acid                   | NT | 33.5 ± 3.1 | 71.2 ± 4.9 | 85.5 ± 2.8b | 92.7 ± 2.4a | 94.1 ± 2.1a |

*<sup>b</sup>*β-carotene-linoleic acid assay. NT: not tested. In a column, means ± SD with same letter notification are not significantly different at (p < 0.05) (n = 3).
diterpenes were isolated from different *Premna* species and were found to be responsible for the cytotoxic activity (Hymavathi et al. 2009; Suresh et al. 2011; Naidu et al. 2014). The diterpenes latifoliol, dihydrolatifoliol and latiferanol which were isolated from the stem-bark of *P. latifolia* Roxb., were found to be effective against colon (HT-29), skin (A-431), breast (MCF-7), liver (Hep-G2), prostate (PC-3), lung (A-549), mouse melanoma (B-16 F10) and kidney (ACHN) cancer cell lines in vitro with IC50 values ranging from 0.02 to 58.24 µg/mL (Salae & Boonnak 2013). We think that the the n-hexane fraction is rich in diterpenoid derivatives but further isolation work is still needed. Our data showed that also the isolated flavonoids from dichloromethane/ethyl acetate fraction, seems to have cytotoxic activity. These results are in agreement with several published data. Quercetin isolated from *Athrixia phyllicoides* DC. (Asteraceae) exhibited cytotoxic activity with IC50 value of 81.38 µg/mL against African green monkey kidney (Vero) cell line (Mavundza et al. 2010). Moreover, quercetin exhibited potent growth inhibitory activity against human epidermoid carcinoma KB and KBv200 cells (Zhang et al. 2013). In addition, quercetin was found to induce apoptosis in KB and KBv200 cells via the mitochondrial pathway, including a decrease of the reactive oxygen species level, loss of mitochondrial membrane potential, release of cytochrome c into cytosol and significant activation of caspase-9 and caspase-3, and cleavage of poly (ADP-ribose) polymerase (Zhang et al. 2013). 3-Methoxy quercetin which was isolated from *Inula viscosa* showed also cytotoxic activity against larynx carcinoma (Hep-2) and breast epithelial adenocarcinoma (MCF-7) cell lines with IC50 values of 26.12 and 11.23 µg/mL, respectively (Talib et al. 2012). Kaempferol has been found to posses antiinflammatory properties through the induction of the apopitosis. The treatment of the chronic myelogenous leukemia cell line K562 and promyelocytic human leukemia U937 with kaempferol caused an induction of apoptosis by decreasing the expression the anti-apoptotic protein Bcl-2 and increasing the expressions of the pro-apoptotic protein Bax. Furthermore, kaempferol has been shown to induce mitochondrdial release of cytochrome c into cytosol and significant activation of caspase-3, and -9 as well as PARP cleavage (Marfe et al. 2009).

The antioxidant activity of *P. resinoso* crude extract and its fractions in our study is in agreement with recent reports that have shown the antioxidant activity of several *Premna* species including *P. serratifolia*, *P. tomentosa*, and *P. esculenta* Roxb. (Mahmud et al. 2012; Selvam et al. 2012; Naidu et al. 2014). The ethanol and aqueous extracts of *P. serratifolia* have been reported to have promising free radical scavenging activity of DPPH compared to that of ascorbic acid. The scavenging activity of ethanol extract was higher than the aqueous extract (Rajendran et al. 2009).

In general, the antioxidant activity of plant phenolics including flavonoids, resides mainly in their ability to donate hydrogen atoms or electrons and thereby scavenge the free radicals (Sharififar et al. 2009). Flavonoids are known as important secondary plant products in foods and medicinal plants. They have attracted much attention in relation to their wide range of activities in the prevention of cancer, inflammation and coronary heart disorders (Garcia-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, Tuñón MJ, Gonzalez-Gallego J. 2007. The anti-inflammatory flavonoids quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. Eur J Pharmacol. 557:221–229).

In conclusion, the results of the present study revealed antimicrobial, cytotoxic and antioxidant activities of *Premna resinosa* crude methanol extract and its fractions. Bioactivity-guided fractionation of the most active dichloromethane/ethyl acetate and n-hexane fractions led to isolation of five flavonoids, namely, quercetin, 3-methoxy quercetin, kaempferol, 3-methoxy kaempferol, myricetin 3,7,3′-trimethyl ether as well as lupeol and stigmasterol for the first time from this species. Further studies should be carried out to isolate and characterize other active compounds and to determine the mechanism of the pleotropic activities of *P. resinosa*.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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