Saudi Pharmaceutical Journal 27 (2019) 162–170

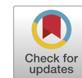
Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Comparative evaluation of cytotoxic, antimicrobial and antioxidant activities of the crude extracts of three *Plectranthus* species grown in Saudi Arabia



Ramzi A. Mothana [a,*], Jamal M. Khaled [b], Ali A. El-Gamal [a], Omar M. Noman [a], Ashok Kumar [c], Mohamed F. Alajmi [a], Adnan J. Al-Rehaily [a], Mansour S. Al-Said [a]

[a] *Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[b] *Departments of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[c] *Vitiligo Research Chair, College of Medicine, King Saud University, Riyadh, Saudi Arabia*

ARTICLE INFO

Article history:
Received 10 June 2018
Accepted 20 September 2018
Available online 25 September 2018

Keywords:
Plectranthus species
Diterpenoids
Cytotoxic
Antimicrobial
Antioxidant

ABSTRACT

Natural products from medicinal plants represent major resource of novel therapeutic substances for combating serious diseases including cancers and microbial infections. The genus *Plectranthus* (Family: Labiatae) represents a large and widespread group of species with a diversity of traditional uses in treatment of various ailments. Therefore, this research study aimed to evaluate the cytotoxic, antimicrobial and antioxidant activities of three *Plectranthus* species growing in Saudi Arabia namely *P. cylindraceus* Hocst. ex Benth., *P. asirensis* JRI Wood and *P. barbatus* Andrews. Moreover, this work focused on the isolation of the active constituents responsible for the activities from the most active *Plectranthus* species.

The extracts were tested for their cytotoxic activity against three cancer cell lines (Hela, HepG2 and HT-29), using MTT-test, antimicrobial activity against Gram positive, Gram negative bacterial and fungal strains using broth micro-dilution assay for minimum inhibitory and bactericidal concentrations (MIC and MBC) and antioxidant activity using scavenging activity of DPPH radical and β-carotene-linoleic acid methods. The ethanolic extracts of the *Plectranthus* species showed remarkable cytotoxic activity against all cancer cell lines with IC_{50} values ranging between 10.1 ± 0.33 to 102.6 ± 8.66 μg/mL and a great and antimicrobial activity with MIC values between 62.5 and 250 μg/mL. In addition, the three *Plectranthus* species showed almost moderate antioxidant activity. The most interesting cytotoxic and antimicrobial results were observed with the extract of *P. barbatus*. Consequently, this extract was partitioned between water and *n*-hexane, chloroform and *n*-butanol and tested. The cytotoxic activity resided predominantly in the *n*-hexane and chloroform fractions. The analysis of the chloroform fraction led to the isolation of four diterpenoid compounds, two of labdane- and two of abietane-type, which were identified as coleonol B, forskolin, sugiol and 5,6-dehydrosugiol. Purification of the *n*-hexane fraction led to isolation of a major abietane-type diterpene, which was identified as ferruginol. Sugiol, 5,6-dehydrosugiol and ferruginol were isolated for the first time from *P. barbatus* in this study. The isolated diterpenoids showed variable cytotoxic effects with IC_{50} values between 15.1 ± 2.03 and 242 ± 13.3 μg/mL, a great antimicrobial activity with MIC values between 15.6 and 129 μg/mL and a total antioxidant activity ranging from 23.1 ± 2.9 to 69.2 ± 3.8%.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Medicinal plants continue to be an important resource to fight serious diseases, especially in developing countries (Alves-Silva et al., 2017; Ngezahayo et al., 2015). Malignant and infectious diseases are still a serious problem to public health, despite the great development in modern medicine.

Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6

* Corresponding author.
E-mail address: rmothana@ksu.edu.sa (R.A. Mothana).

Peer review under responsibility of King Saud University.

https://doi.org/10.1016/j.jsps.2018.09.010
1319-0164/© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

deaths is due to cancer (WHO, 2014; WHO, 2017). Approximately 70% of deaths from cancer occur in low- and middle-income countries (Aidi Wannes et al., 2017; WHO, 2014; WHO, 2017).

In addition, the treatment of infectious diseases caused by resistant bacterial strains, represent one of the main challenges of medicine today, especially due to the inefficacy of long-term drug therapy (Aldulaimi, 2017). The relative unavailability of medicines in developing countries and the appearance of widespread multiresistant bacterial strains let the effect of these diseases particularly large and considerable (Ngezahayo et al., 2015; Okeke et al., 2005). In the search for new alternatives to treat these infections, many researchers have been looking for novel compounds derived from natural products to replace, or be used in combination with conventional antibiotics (Aldulaimi, 2017; Cowan, 1999; Enioutina et al., 2017). Consequently, medicinal plants have served as an important source of effective antimicrobial and anticancer agents. Over 60% currently used anticancer agents were isolated from natural sources, including plants, marine organisms and microorganisms or are related to them (Sultana et al., 2014; Newman et al., 2003). These include the naturally derived taxanes e.g. paclitaxel isolated from *Taxus baccata* and *Taxus brevifolia*, etoposide and teniposide, the semi-synthetic derivatives of epipodophyllotoxin, isolated from species of the genus *Podophyllum*, the Vinca alkaloids, vinblastine and vincristine, isolated from *Catharanthus roseus*, the semisynthetic derivatives of camptothecin, irinotecan and topotecan, isolated from *Camptotheca acuminata*, and several others (Cragg and Newman, 2000; Wang et al., 2014). Thus, the interest in the use of folk medicine for tumor treatment or prevention has increased (Mbaveng et al., 2017; Mothana et al., 2007; Wong et al., 2017).

Generally, considerable part of the research performed today focuses on the development of new drugs to treat microbial infections as well as cancers and other diseases (Aldulaimi, 2017; Enioutina et al., 2017; Mbaveng et al., 2017). Therefore, this work aimed to evaluate the antimicrobial, anticancer and antioxidant activities of selected medicinal plants of the genus *Plectranthus* grown in Saudi Arabia.

The genus *Plectranthus* (Family: Lamiaceae) represents a large and widespread group of species with a diversity of traditional uses. This genus comprises a group of around 300 species, distributed in tropical and suptropical areas of Africa, Asia and Australia (Abdel Khalik, 2016; Amina et al., 2017; Lukhoba et al., 2006). The *Plectranthus* genus is represented in Saudi Arabia by seven species distributed in the South of the Kingdom (Abdel Khalik, 2016). The *Plectranthus* species are notable medicinal plants utilized broadly for the treatment of different sicknesses. A diversity of traditional medicinal uses of *P. barbatus* in India (Hindu and Ayurvedic medicine), Africa, China, and South America have been reported. Most of these plant species are utilized for intestinal disturbance and respiratory disorders, heart diseases, liver fatigue, malaria and certain central nervous system disorders (Alasbahi and Melzig, 2010; Lukhoba et al., 2006; Viswanathaswamy et al., 2011). *Plectranthus* species are rich in diterpenoids of different types particularly the abietane-type which are reported to be responsible for various pharmacological activities such as antibacterial and antifungal (Abdissa et al., 2017; Gaspar-Marques et al., 2006; Stavri et al., 2009; Van Zyl et al., 2008), cytotoxic (Amina et al., 2017; Yulianto et al., 2016) and antiplasmodial (Alegre-Gómez et al., 2017; Mothana et al., 2014) activities. The present project is a part of our ongoing studies on the biological activities of Saudi medicinal herbs and aims to provide data on the anticancer, antimicrobial activities as well as on the antioxidant potential of three *Plectranthus* species namely *P. cylindraceus* Hocst. ex Benth., *P. asirensis* JRI Wood and *P. barbatus* Andrews. Moreover, this research project focused on the isolation of the active constituents responsible for the activities from the most active *Plectranthus* species.

2. Experimental

2.1. Plant materials

Aerial parts of *P. cylindraceus, P. asirensis* were collected from Shatha Al-Ala mountain in Al-Baha region while *P. barbatus* was collected from Al-Namas area in Asir-region., Saudi Arabia in December 2016 and identified at the Pharmacognosy Department, College of Pharmacy, King Saud University (KSU). Voucher specimen were deposited at the Pharmacognosy Department, College of Pharmacy, King Saud University.

2.2. Extraction of plant materials

The air-dried and powdered aerial parts of the plant species were extracted with 96% ethanol using maceration. The obtained ethanolic extracts were filtered and evaporated by using a rotary evaporator or freeze dryer and kept at 4 °C until testing. The most antimicrobial and cytotoxic active ethanolic extract was then suspended in water and fractionated with different organic solvents e.g. *n*-hexane, chloroform and *n*-butanol. The obtained fractions were filtered, evaporated by using a rotary evaporator or freeze dryer and kept at 4 °C until testing.

2.3. Fractionation and isolation of active principles

The air-dried and powdered aerial parts of the three *Pleactranthus* species of (1 kg of each) were extracted by maceration with 96% ethanol (5 × 2 L) at room temperature. The combined obtained ethanolic extracts were filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator. Each extract was tested for its cytotoxic, antimicrobial and antioxidant activities. The ethanolic extract of *P. barbatus* showed the greatest activities in comparison to the extracts of *P. cylindraceus, P. asirensis*. Consequently, *P. barbatus* was chosen for further investigations, fractionation and isolation. Thus, part of the dried ethanolic extract (40 g) was subsequently redissolved in 30% ethanol (300 mL) and partitioned successively for several times with *n*-hexane (3 × 250 mL), chloroform (3 × 250 mL) and *n*-butanol (3 × 250 mL) to provide the corresponding extracts. Of these fractions, the *n*-hexane and chloroform fractions exhibited interesting antimicrobial, anticancer and antioxidative activities. Therefore, part of *n*-hexane- (5 g) and chloroform-fractions (3 g) were subjected to different chromatographic techniques in order to isolate the responsible active compounds.

The chloroform-fraction was separated on column chromatography on a pre-packed silica gel column (35 mm i.d. × 350 mm) to give 10 fractions. The elution was performed with a gradient of chloroform:methanol, to pure methanol. TLC analysis of the fractions with anisaldehyd/sulfuric acid and heating at 100 °C allowed the constitution of 10 fractions.

Fraction 7 was separated on a RP18 column with methanol: water (1:9) (20 mm i.d. × 700 mm) to afford four subfractions (Fraction 7a, 7b, 7c and 7d). Fraction 7d afforded a crystalline compound 1 (**Pb1**) (10 mg). Fraction 5 was subjected to a silica gel column chromatography using a gradient of dichlormethane (DCM): methanol (MeOH) (100:1) as a solvent to afford three subfractions (fraction 5a, 5b and 5c) Fraction 5c afforded compound 2 (**Pb2**) which required a further separation on the chromatotron (centrifugal TLC) (silica gel 60, 0.04–0.06 mm, 1 mm and MeOH:DCM, 0.5:99.5) to give crystalline compound 2 (Pb2) (11 mg).

Fraction 3 was separated on a silica gel column with a gradient of hexane:acetone as eluent to afford four subfraction (Fractions 3a, 3b, 3c and 3d). Fraction 3a which required a separation on chromatotron (Silica gel, 0.04–0.06 mm, 1 mm, with hexane:ethyl

acetate (EtoAc), 1:1) gave compound 3 (**Pb3**) (14 mg). Fraction 3c contained a major compound which was compound 4 (**Pb4**) that needed a further purification on a chromatotorn (silica gel 60, 0.04–0.06 mm, 1 mm and EtoAc:DCM, 1:9) to give compound 4 (**Pb4**) (17 mg).

Compound 5 (**Pb5**) was obtained from the *n*-hexane-fraction which was separated on column chromatography on a pre-packed silica gel column (35 mm i.d. × 350 mm) to give 15 fractions. The elution was performed with a gradient of hexane:EtoAc to pure EtoAc. Compound 5 (**Pb5**) represented the major compound in fraction 3. The purification of this fraction on a silica gel column with hexane: EtoAc (3:1) as eluent gave a viscous compound 5 (**Pb5**) (90 mg).

2.4. Elucidation of the chemical structure

The UV and IR spectra were recorded on Hitachi-UV-3200 and JASCO 320-A spectrometers. The ^{1}H-, ^{13}C NMR and 2D-NMR spectra were recorded on a Bruker AMX-500 spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are given in ppm relative to tetramethylsilane internal standard. Jeol JMS-700 High Resolution Mass Spectrophotometer was utilized for the mass determination. Electron impact mode of ionization was used, keeping ionization energy of 70ev. Resolution was set up to 10 k direct probe was used with temperature ramp setting, initial temperature 50 °C rise with rate of 32 °C per minute and final temperature set up to 350 °C. Thin layer chromatography (TLC) was carried out on precoated silica gel F254 plates (E. Merck, Darmstadt, Germany); detection was performed at 254 nm and by spraying with p-anisaldehyde/H_2SO_4 reagent. All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.5. Determination of cytotoxic activity on human cancer cell lines

2.5.1. Cancer cell lines and culture

The study was performed on three tumor cell lines, human cervical cancer (HeLa), human hepatocellular liver carcinoma (HepG2) and human colon cancer (HT-29). HeLa, HepG2 and HT-29 cells were maintained in DMEM/high glucose supplemented with 2 mM l-glutamine, 10% fetal calf serum and 1% penicillin-streptomycin.

2.5.2. In vitro antiproliferative activity by MTT test

The cytotoxic activity of the crude extracts, fractions and isolated compounds was assessed on cell viability utilizing MTT-test. This assay measures the cellular viability based on reduction capacity of the viable cells to convert 3-[4,5-dimethylthiazol-2-y l]-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals as previously described (Albadawi et al., 2017) with some modifications. Briefly, cells were seeded (2 × 10^4 cells/well) in growth medium (DMEM) into flat-bottom microdilution plates of 96 wells (in quintuplicates) and incubated at 37 °C in a 5% CO_2 incubator for 24 h. The extracts or compounds were added at different concentrations to each well while the medium in control wells was replaced by SFM (serum free medium) containing an equivalent volume of dimethyl sulfoxide (1% DMSO) and incubated for further 24 h. The concentrations tested ranged from 7.8 to 500 μg/ml. After that SFM was removed and 100 μl of MTT (0.5 mg/ml) was added to each well and incubated at 37 °C for another 3 h to estimate cell viability. After removing the MTT solution 100 μl isopropanol was added to each well and shacked for 1 h at room temperature. At the end, the plates were read at 549 nm utilizing a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT, USA). By comparing the optical density (OD) values against those in control wells, the induced cytotoxicity was calculated. Cytotoxicity was expressed as IC_{50}-value which was calculated as the concentration of the extract inhibiting cell viability by 50%. Dasatinib was used as a positive control. All measurements were performed in triplicate and the means and standard errors were calculated.

2.6. Determination of antimicrobial activity

2.6.1. Test microorganisms

The bacterial and fungal strains utilized in this study were *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 25175), *Escherichia coli* (ATCC 35218), *Salmonella typhi* wild strain, *Aspergillus niger* (AUMC 8777), *Penicillium aurantiogriseum* (AUMC 9302), *Candida albicans* (ATCC 60193) and *Cryptococcus neoformans* wild strain.

2.6.2. Minimum inhibitory concentrations (MIC)

The MICs of the crude extracts, fractions and isolated compounds against two Gram-positive, two Gram-negative and four fungi strains were determined utilizing micro-well dilution method as described previously by Mann and Markham (1998) with modifications. With sterile round-bottom 96-well plates, duplicate two-fold serial dilutions of each sample (100 μl/well) were made in the suitable broth (Mueller Hinton or Sabouraud Dextrose broth) containing 5% (v/v) DMSO to establish a range of concentrations of samples (2000 to 31.2 μg/ml for extracts and 250 to 3.9 μg/ml for compounds). 100 μl (1 × 10^6 CFU/ml) of the bacterial or fungal suspension which was previously prepared in the proper broth, was then added in each well except those in column 10, 11 and 12, which used as negative controls for extract, saline and media sterility. The last well in each plate was served for bacterial or fungal growth without sample. After that the bacterial plates were incubated at 37 °C for 24 h while fungal plates were incubated at 25 °C for 72 h. The MIC of each sample was determined as the lowest concentration showing no detectable bacterial or fungal growth. Two positive controls were utilized namely, gentamycin and nystatin. For the estimation of MBC and MFC, 5 μl of the liquid from the wells that showed no growth was taken and incubated on agar plates for further 24 or 72 h. MBC and MFC are the lowest concentrations that evidenced no visible bacterial or fungal growth.

2.7. Determination of antioxidant activity

2.7.1. DPPH radical-scavenging activity

The antioxidant activity of the extracts, fractions and isolated compounds was estimated utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described previously by Brand-Williams et al. (1995). This test measures the radical scavenging activity of the radicle DPPH by the investigated samples. Five concentrations (10, 50, 100, 500 and 1000 μg/ml) of each sample were prepared. Briefly, 500 μl of each sample was added to 125 μl DPPH methanol solution (1 mM) and 375 μl methanol and incubated for 30 min at room temperature in the dark. Ascorbic acid was used as a positive control. After that, the DPPH radical-scavenging activity was determined by measuring the absorbance at λ = 517 nm and calculated using the equation:

$$\% \text{ of anti} - \text{radical activity} = Abs_{control} - Abs_{sample} / Abs_{control} \times 100$$

The experiments were performed in triplicate and the means and standard errors were calculated.

2.7.2. β-Carotene bleaching assay

The antioxidant activity of the extracts, fractions and isolated compounds was determined by utilizing the β-carotene bleaching assay as previously described by Mohd-Esa et al., (2010) with modification. β-carotene solution (1000 μl) which was ready made by dissolving 200 μg in 1 mL chloroform, was added to a flask

containing a solution of 200 μl of Tween-20 and 20 μl of linoleic acid. The chloroform was removed from the mixture utilizing rotatory evaporator and then 100 mL of distilled water was added to the mixture and vigorously shaken for 2 min. 200 μl of each sample (1000 μg/ml) was added to 2 mL of the β-carotene-linoleic acid emulsion and incubated at 40 °C for 2 h. Finally the absorbance was measured at 470 nm at 30 min intervals, using a UV-spectrophotometer (UV mini-1240, Shimadzu, Japan). Rutin at a concentration of 1000 μg/ml was utilized as a positive control. The antioxidative activity was calculated by using the following formula:

$$\text{Antioxident activity}\,(\%) = 1 - (\text{Abs0} - \text{Abst})/(\text{Abs}^{\circ}0 - \text{Abs}^{\circ}t) \times 100$$

where Abs0 and Abs°0 are the absorbencies measured at zero time of incubation for the sample and blank, respectively. Abst and Abs°t are the absorbencies for sample and blank, respectively, at 120 min. All measurements were performed in triplicate and the means and standard errors were calculated.

3. Results

In our continuing search for bioactive compounds from medicinal plants grown in Saudi Arabia, this comparative investigation was carried to evaluate the anticancer, antimicrobial and antioxidant activities of three *Plectranthus* species namely *P. cylindraceus, P. asirensis* and *P. barbatus*.

3.1. Cytotoxic activity

As demonstrated in Table 1, the crude ethanolic extracts of the *Plectranthus* species showed remarkable cytotoxic activity against all cancer cell lines with IC_{50} values ranging between 10.1 and 102.6 μg/mL. The most interesting cytotoxic results against Hela, HepG2 and HT-29 cancer cell lines were observed with the extract of *P. barbatus* with IC_{50} values of 10.1, 10.7 and 32.0 μg/mL, respectively which were comparable with the positive control values (Table 1). Consequently, this extract was chosen for bioassay-guided fractionation on the basis of its cytotoxic effect. The extract of *P. barbatus* was partitioned between water and *n*-hexane, chloroform and *n*-butanol and tested. The cytotoxic activity resided predominantly in the *n*-hexane and chloroform fractions with IC_{50} values between 12.8 and 27.8 μg/mL (Table 1). *n*-Butanol showed only a moderate activity with IC_{50} values between 45.7 and 55.2 μg/mL. Thus, *n*-hexane, chloroform extracts were selected and subjected to column chromatography to isolate the responsible compounds.

Four compounds were isolated from chloroform fraction namely compounds Pb1-Pb4 (Coleonol B, forskolin, sugiol and 5,6-dehydrosugiol) and one compound Pb5 (ferruginol) from hexane-fraction. The isolated diterpenoids showed interesting cytotoxic effects with IC_{50} values between 15.1 and 242 μg/mL (Table 1). Table 1 showed that the greatest activity was shown by ferruginol (IC_{50}: 15.1–26.5 μg/mL), followed by 5,6-dehydrosugiol (IC_{50}: 41.2–69.5 μg/mL) and forskolin (IC_{50}: 40.7–72.7 μg/mL). Sugiol and Coleonol B exhibited a moderate to weak cytotoxic activity (IC_{50}: 70.5–242.2 μg/mL).

Table 1
Cytotoxic activity of the crude extracts of *Plectranthus* species and fractions and isolated compounds from *P. barbatus* (μg/mL).

Extracts and fracions	IC_{50} values (μg/mL)		
	Hela	HepG2	HT-29
P. cylindraceus	65.94 ± 3.92	69.89 ± 4.16	102.60 ± 8.66
P. asirensis	114.4 ± 8.44	87.91 ± 17.74	99.16 ± 12.15
P. barbatus	10.16 ± 0.33	10.72 ± 0.8	32.06 ± 1.92
n-Hexane fraction	12.82 ± 0.39	13.02 ± 0.93	27.33 ± 0.26
Chloroform fraction	26.84 ± 0.63	27.82 ± 1.90	25.93 ± 3.02
n-Butanol fraction	53.64 ± 1.89	55.29 ± 2.60	45.78 ± 1.03
Pb 1 (coleonol B)	70.50 ± 5.15	82.70 ± 1.6	242.23 ± 13.3
Pb 2 (forskolin)	58.15 ± 2.73	72.75 ± 2.91	40.72 ± 3.63
Pb 3 (sugiol)	94.99 ± 7.88	97.61 ± 28.9	85.57 ± 16.52
Pb 4 (5,6-dehydro sugiol)	41.23 ± 1.1	69.55 ± 1.36	59.26 ± 2.87
Pb 5 (ferruginol)	15.10 ± 2.03	26.58 ± 0.48	25.98 ± 4.23
Dasatamib	26.16 ± 1.17	6.31 ± 0.87	28.19 ± 1.02

3.2. Antimicrobial activity

MIC, MBC and MFC values are demonstrated in Table 2. As shown in Table 2, the most sensitive strains were the Gram-positive bacteria *Streptococcus mutans* and the fungal strain *Cryptococcus neoformans* which were more susceptible to *Plectranthus* extracts with MIC values between 62.5 and 250 μg/mL than the other microbial strains. Minimum bactericidal (MBC) or minimum fungicidal effects (MFC) were also exhibited and obtained one time higher than that of MIC's with all extracts. Again *P. barbatus* crude extract appeared to be highly effective against all microorganisms and was the strongest among the three plants (Table 2). Thus, its *n*-hexane, chloroform and *n*-butanol fractions were tested. Table 2 showed that the antimicrobial activity resided predominantly in the *n*-hexane and chloroform fractions with MIC values between 78.1 and 156.2 μg/mL. *n*-Butanol fraction showed only a weak antimicrobial activity.

In addition, all 5 isolated compounds showed a variable antimicrobial activity against the microorganisms tested (Table 3). The greatest antimicrobial activity was observed with Pb1, Pb2 and Pb3 (Coleonol B, forskolin, sugiol) with MIC values between 15.6 and 31.5 μg/mL (Table 3).

3.3. Antioxidant activity

The results of the antioxidant activity are presented in Table 4. In the β-carotene-linoleic acid model system, the three *Plectranthus* extracts showed the ability to inhibit the discoloration of β-carotene at a concentration of 1000 μg/mL with total antioxidant value of 71, 65 and 66% for *P. asirensis*, *P. cylindraceus* and *P. barbatus* respectively (Table 4). In addition, results of the DPPH radical scavenging method demonstrated comparable free radical scavenging activity for the three *Plectranthus* species (Table 4). *P. asirensis*, *P. cylindraceus* and *P. barbatus* showed at 500 and 1000 μg/mL scavenging activity between 80 and 88%. In addition to that, the chloroform fraction of *P. barbatus* exhibited the highest antioxidant and free radical scavenging activity among the tested fractions with values of 76 and 80% at 1000 μg/mL (Table 4). Among the isolated compounds, compounds Pb3, Pb4 and Pb5 (sugiol, 5,6-dehydrosugiol and ferruginol) were able to inhibit the discoloration of β-carotene with total antioxidant values of 65, 67, 69% respectively (Table 4).

3.4. Structure elucidation of the isolated compounds

Since the investigated activities remained principally in the chloroform and hexane fractions of *P. barbatus* extract, these fractions were subjected to various chromatographic separation techniques. The analysis of the chloroform fraction led to the isolation of four diterpenoid compounds, two of labdane- and two of abietane-type, which were identified as coleonol B (**compound Pb1**), forskolin (**compound Pb2**), sugiol (**compound Pb3**) and 5,6-dehydrosugiol (**compound Pb4**). Whereas the fractionation and purification of the *n*-hexane fraction led to isolation of one major abietane-type diterpene, which was identified as ferruginol (**compound Pb5**). Sugiol, 5,6-dehydrosugiol and ferruginol were isolated for the first time from *P. barbatus* in the present study.

Table 2
Minimal inhibitory concentrations, minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) of the ethanolic extracts of *Plectranthus* species and fractions of *P. barbatus* (μg/mL).

Microorganisms		Activity	*P. cylindraceus*	*P. asirensis*	*P. barbatus*	Hexane Fr.	Chloroform Fr.	Butanol Fr.	RA*	RA**
Bacteria	*S. aureus*	MIC	250	250	125	78.12	78.12	312.5	7.8	NT
		MBC	500	500	125	156.25	156.25	625	15.6	NT
	S. mutans	MIC	125	250	62.5	78.12	78.12	312.5	7.8	NT
		MBC	250	500	125	156.25	156.25	1250	15.6	NT
	S. typhi	MIC	250	250	125	156.25	78.12	625	3.9	NT
		MBC	500	500	250	312.5	156.25	1250	7.8	NT
	E. coli	MIC	250	500	250	156.25	78.12	625	3.9	NT
		MBC	500	1000	500	312.5	156.25	1250	7.8	NT
Fungi	*A. niger*	MIC	500	250	125	156.25	156.25	625	NT	3.5
		MFC	1000	500	250	312.5	312.5	1250	NT	7.0
	C. albicans	MIC	500	500	250	312.5	156.25	625	NT	3.5
		MFC	1000	1000	500	625	312.5	1250	NT	7.0
	P. aurantiogriseum	MIC	500	500	125	156.25	156.25	625	NT	7.0
		MFC	1000	1000	250	312.5	312.5	2500	NT	14.0
	C. neoformans	MIC	125	125	62.5	78.12	78.12	312.5	NT	1.75
		MFC	250	250	125	156.12	78.12	625	NT	3.5

Staphylococcus aureus ATCC 25923, Streptococcus mutans ATCC 25175, Escherichia coli ATCC 35218, Salmonella typhi wild strain, Aspergillus niger AUMC 8777, Penicillium aurantiogriseum AUMC 9302, Candida albicans ATCC 60193 and Cryptococcus neoformans wild strain, RA*: Reference antibiotic (Gentamycin), RA**: Reference antibiotic (Nystatin). NT: not tested.

Table 3
Minimal inhibitory concentrations, minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) of the isolated compounds from the active *P. barbatus* fractions (μg/mL).

Microorganisms			Isolated Compounds					References	
		Activity	Pb 1	Pb 2	Pb 3	Pb 4	Pb 5	RA*	RA**
Bacteria	*S. aureus*	MIC	15.6	15.6	15.6	31.25	31.25	7.8	NT
		MBC	31.25	31.25	31.2	64.5	64.5	15.6	NT
	S. mutans	MIC	15.6	15.6	15.6	31.25	31.25	7.8	NT
		MBC	31.25	31.25	31.25	64.5	64.5	15.6	NT
	S. typhi	MIC	15.6	15.6	31.25	64.5	64.5	3.9	NT
		MBC	31.25	31.25	64.5	129	129	7.8	NT
	E. coli	MIC	31.25	31.25	31.25	64.5	64.5	3.9	NT
		MBC	64.5	64.5	31.25	129	129	7.8	NT
Fungi (Yeasts and molds)	*A. niger*	MIC	31.25	31.25	31.25	64.5	64.5	NT	3.5
		MFC	64.5	64.5	64.5	129	129	NT	7.0
	C. albicans	MIC	64.5	64.5	64.5	64.5	64.5	NT	3.5
		MFC	129	129	129	129	129	NT	7.0
	P. aurantiogriseum	MIC	31.25	31.25	31.25	64.5	64.5	NT	7.0
		MFC	64.5	129	64.5	129	129	NT	14.0
	C. neoformans	MIC	15.6	15.6	31.25	31.25	31.25	NT	1.75
		MFC	31.25	31.25	64.5	64.5	64.5	NT	3.5

Staphylococcus aureus ATCC 25923, Streptococcus mutans ATCC 25175, Escherichia coli ATCC 35218, Salmonella typhi wild strain, Aspergillus niger AUMC 8777, Penicillium aurantiogriseum AUMC 9302, Candida albicans ATCC 60193 and Cryptococcus neoformans wild strain, RA*: Reference antibiotic (Gentamycin), RA**: Reference antibiotic (Nystatin). NT: not tested.

Table 4
Antioxidant activity and free radical scavenging activity of the extracts of *Plectranthus* species, fractions and isolated compounds of *P. barbatus*.

Samples	Total antioxidant Activity in % (1000 μg/ml)	Free Radical Scavenging Activity in % (DPPH assay)				
		10	50	100	500	1000
		(μg/mL)				
P. asirensis	71.6 ± 5.1	28 ± 3.1	27.9 ± 3.8	66.9 ± 1.6	86.5 ± 2.1	85.1 ± 1.3
P. cylindraceus	65.7 ± 3.8	48.2 ± 5.1	67.2 ± 4.1	80.4 ± 3.3	84.3 ± 3.2	88.3 ± 3.0
P. barbatus	66.3 ± 2.1	15.8 ± 3.6	50.5 ± 2.2	80.2 ± 1.1	80.6 ± 1.8	81.2 ± 1.1
n-Hexane fraction	38.0 ± 1.8	12.8 ± 1.5	14.6 ± 2.0	22.0 ± 2.9	32.4 ± 2.8	47.6 ± 3.4
Chloroform fraction	76.0 ± 4.9	25.6 ± 2.4	31.2 ± 3.5	50.8 ± 4.6	76.2 ± 5.4	80.1 ± 5.9
n-Butanol fraction	68 ± 3.1	17.4 ± 1.6	22.4 ± 1.2	35.2 ± 2.0	60.8 ± 3.2	71.9 ± 3.8
Pb1 (Coleonol B)	23.1 ± 2.9	NT	NT	NT	NT	NT
Pb2 (forskolin)	25.9 ± 2.7	NT	NT	NT	NT	NT
Pb3 (sugiol)	64.8 ± 3.6	NT	NT	NT	NT	NT
Pb4 (5,6-dehydrosugiol)	67.4 ± 4.1	NT	NT	NT	NT	NT
Pb5 (ferruginol)	69.2 ± 3.8	NT	NT	NT	NT	NT
Ascorbic acid	NT	89.6 ± 3.1	89.7 ± 4.9	90 ± 2.8	90.3 ± 2.4	94.4 ± 2.1
Rutin	87.1 ± 3.0	NT	NT	NT	NT	NT

NT: not tested.

The isolated diterpenoids were identified by comparison their spectral data with those reported in the literature (Fig. 1, Table 5). The identification of the isolated compounds was carried out using different spectroscopic techniques including ^{1}H-, ^{13}C NMR and 2D-NMR (COSY, HSQC and HMBC). Compound Pb1 and compound Pb2 showed very similar spectral NMR data. The EIMS spectrum of both compounds displayed an [M+] ion at *m/z* 410 consistent with the molecular formula $C_{22}H_{34}O_7$. The ^{1}H NMR spectrum showed signals for five tertiary methyl groups, a vinyl group linked to a fully substituted to a fully substituted carbon and two geminal protons adjacent to carbonyl (Table 5). The ^{13}C NMR and DEPT spectra revealed the presence of 22 carbons, including five methyl carbons, two carbonyl, two olefinic, and three oxygnated carbons, which suggested the presence of a diterpenoid of labdane-type with acetyl-group (Table 5) (Saksena et al., 1985; Prakash et al., 1988). Comparison of the ^{1}H-, ^{13}C NMR data of both compounds with published data showed that compound Pb2 and compound Pb3 are Coleonol B and forskolin which were previously isolated from different Labiatae species including *P. barbatus* (Saksena et al., 1985; Prakash et al., 1988).

The mass data of compounds Pb3, Pb4 and Pb5 gave molecular masses of 300, 298 and 286 and the molecular formula $C_{20}H_{28}O_2$, $C_{20}H_{26}O_2$ and $C_{20}H_{30}O$ respectively. ^{1}H-, ^{13}C NMR data are demonstrated in Table 5. Compound Pb3 and compound Pb4 showed very similar spectral NMR data with only one difference which is the presence of two olefinic C-atoms. ^{13}C NMR and DEPT-135 spectra of the three compounds displayed 20 signals which comprised five methylene groups in compound Pb5 and four in compound Pb3 and three in compound Pb4, six quaternary carbon atoms including one carbonyl group in compound Pb3 and Pb4 and nine signals of methyl and methane groups (Table 5). ^{1}H NMR spectrum and COSY correlations showed the presence of a septet signal at 3.1 ppm and pair of doublets for methyl groups at 1.21 and 1.22 ppm indicating the presence of isopropyl group in the three structures (Table 5). The presence of three singlet signals and two doublet signals of methyl groups and the septet signal in the ^{1}H NMR spectrum indicated that compounds Pb3, Pb4 and Pb5 are abietane diterpenoids. Comparison of the ^{1}H-, ^{13}C NMR results of the three compounds with published data showed that compounds Pb3, Pb4 and Pb5 are sugiol, 5,6-dehydrosuiol and ferruginol which

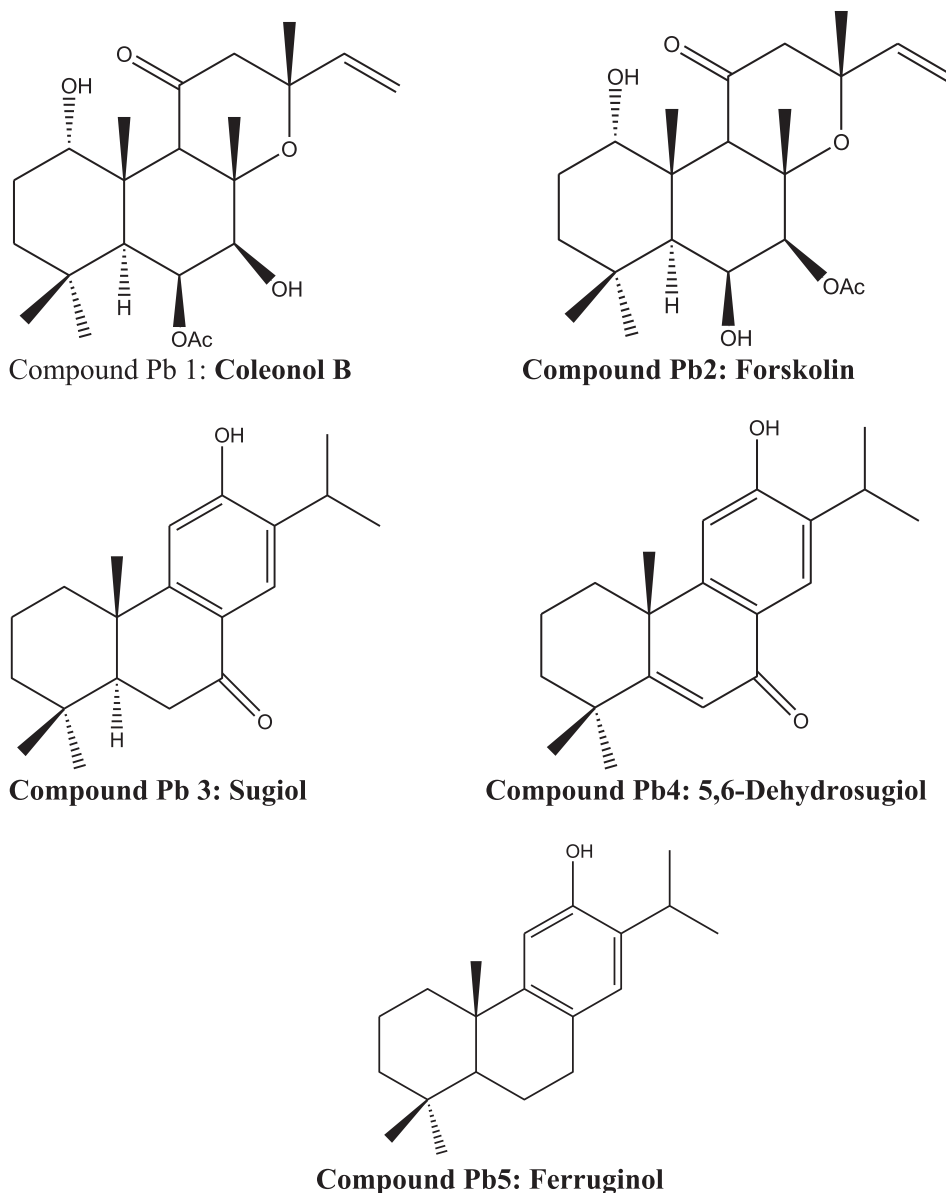


Fig. 1. Chemical structures of the isolated diterpenes from *P. barbatus*.

Table 5
^{1}H and ^{13}C NMR Data of the isolated compounds **Pb1-Pb5** in DMSO.

Position	Comp. 1 (Pb1)		Comp. 2 (Pb2)		Comp. 3 (Pb3)		Comp. 4 (Pb4)		Comp. (Pb5)	
	δ_H	δc	δ_H	δc	δ_H	Δc	δ_H	δc	δ_H	δc
1	4.52 t	74.7	4.51 m	75.0	2.18 m, 148 m	37.4	2.29 m, 1.52 m	37.2	2.15 m 1.36 m	38.8
2	2.13 m, 1.41 m	27.3	2.16 m, 1.42 m	27.6	1.79 m, 1.64 m	18.4	2.05 m, 1.68 m	18.1	1.74 m 1.60 m	19.3
3	1.82 m, 1.10 m	37.8	1.78 m, 1.08 m	37.5	1.50 m, 1.23 m	40.7	1.63 m, 1.35 m	40.6	1.47 m, 1.20 m	41.6
4	–	43.9	–	35.3	–	35.4	–	36.9	–	33.6
5	2.34 d	43.7	2.15 t	44.2	1.75 dd	49.0	–	172.2	1.35 dd	50.5
6	5.79 dd,	73.8	4.49 m	69.4	1.48 dd, 1.25 dd	42.5	6.24 s	123.2	1.88 m, 1.67 m	19.2
7	4.27 d	74.5	5.30 d	79.2	–	196.4	–	183.2	2.78 m	29.7
8	–	77.0	–	82.2	–	122.5	–	121.8	–	127.3
9	–	83.7	–	84.0	–	155.7	–	153.4	–	148.6
10	–	44.1	–	44.1	–	37.4	–	37.4	–	37.5
11	–	208.5	–	208.5	6.79 s	109.2	6.95 s	110.4	6.63 s	110.9
12	3.24 d 2.40 d	50.3	3.22 d. 2.39 d	48.8	–	160.0	–	159.1	2.45 s	150.6
13	–	77.0	–	76.7	–	132.4	–	133.4	–	131.3
14	6.17 dd, 11.0	110.5	6.09 dd 10.8	110.5	7.66 s	124.9	7.73 s	123.6	6.83 s	126.6
15	5.16 dd, 4.9 dd	148.4	5.15 dd, 4.89 dd	148.1	3.19	26.0	3.18	26.1	3.12	26.8
16	1.40 s	30.6	1.39 s	30.9	1.19 d	21.0	1.18 d	22.1	1.23 d	22.7
17	1.59 s	23.5	1.70 s	24.6	1.20 d	22.1	1.19 d	22.3	1.21 d	22.5
18	1.00 s	23.8	1.05 s	24.7	0.97 s	32.2	1.22 s	32.2	0.97 s	33.3
19	1.06 s	33.3	1.30 s	33.6	0.94 s	22.3	1.40 s	22.5	0.95	21.6
20	1.45 s	22.0	1.46 s	21.5	1.22 s	22.9	1.29 s	28.9	1.15 s	24.8
CH_3CO	2.14 s	20.3	2.08 s	20.2	–	–	–	–	–	–
CH_3CO	–	172.9	–	172.5	–	–	–	–	–	–

were previously isolated from different Labiatae species e.g. *Salvia* species, *Taxodium* species, *Cryptomeria japonica*, *Libocedrus bidwillii* (Hirasawa et al., 2007; Kusumoto et al., 2010; Kolak et al., 2005; Li et al., 2000; Luis and Grillo, 1993; Russell, 1975; Ying, and Kubo, 1991; Yoshikawa et al., 2006). Sugiol, 5,6-dehydrosuiol and ferruginol are reported for the first time from *P. barbatus*.

4. Discussion

Cancers and in addition, diseases caused by microorganisms and parasites are as yet a genuine hazard to the general public, in spite of the colossal advancement in western medicine. Thus, look into on new anticancer and antimicrobial agents from natural sources ought to be proceeded, keeping in mind the end goal to find novel, more successful and more affordable medications. Subsequently, in our continuing search for remarkable and promising natural compounds from Saudi medicinal herbs, this investigation was conveyed. In the present research study, we investigated the crude extracts of three *Plectranthus* species which are *P. asirensis, P. cylindraceus* and *P. barbatus*.

The existing knowledge about the cytotoxic, antimicrobial and antioxidative activities of the ethanolic extracts of the *Plectranthus* species grown in Saudi Arabia is in many cases limited.

The plant species of the genus *Plectranthus* are long-familiar medicinal species which are widely used for the treatment of various diseases. The majority of uses are for intestinal disturbance, liver fatigue, respiratory disorders, heart diseases, malaria, and certain central nervous system disorders (Alasbahi and Melzig, 2010). In fact, *Plectranthus* species are rich in two groups of natural compounds, namely diterpenoids and volatile oils. It is important to be mentioned that we focused in this study only on the crude extracts of these *Plectranthus* species that should contain the diterpenoids which are mainly responsible for several observed pharmacological activities e.g. antimicrobial (Abdissa et al., 2017; Burmistrova et al., 2015; Stavri et al., 2009), cytotoxic (Amina et al., 2017; Yulianto et al., 2016) and antiplasmodial (Alegre-Gómez et al., 2017) activities. In the current work, we observed promising cytotoxic, antimicrobial and antioxidant activities for the investigated *P. asirensis, P. cylindraceus* and *P. barbatus*.

Our cytotoxic result of *P. cylindraceus* is in agreement with a recent study done by Amina and co-workers in 2017, who isolated maaliol from the ethanolic extract of *P. cylindraceus* and exhibited the greatest cytotoxic activity among the isolated compounds against MBDMB321, HT1080 and Hela cancer cell lines with IC_{50} values of 28.1, 25.9 and 27.1 μg/ml respectively. In addition, it was previously demonstrated that ethanol extract of *P. cylindraceus* has also antimicrobial activity against Gram-positve bacteria and *Mycobacteria* (Orabi et al., 2000).

As far as we know, this work represents one of the few investigations on the cytotoxic and antimicrobial activities of *P. asirensis* and *P. barbatus* grown in Saudi Arabia. Previous work on *P. barbatus* grown in Brazil indicated that both roots and leaves extracts have a great cytotoxic activity against NCI-H292 cell line with IC_{50} values of 29–45 μg/ml (Costa and Nascimento, 2003). A recent study on South African medicinal plants showed that *P. barbatus* is among the plants which have cytotoxic activity towards drug-sensitive parental CCRF-CEM leukemia cells (Saeed et al., 2016). Moreover, our antimicrobial activity result of *P. barbatus* was in agreement with a previous study done on a Tanzanian *P. barbatus* (Kisangau et al., 2007). It is worth to be mentioned that our results of the cytotoxic, the antimicrobial and antioxidant activities of the investigated *P. asirensis, P. cylindraceus* and *P. barbatus* are also in agreement with literature data found for other *Plectranthus* species e.g. *P. punctatus, P. punctatus, P. ecklonii,* and *P. mollis* (Abdissa et al., 2017; Burmistrova et al., 2015; Joshi, 2014; Yulianto et al., 2016).

Since the crude extract of *P. barbatus* showed the greatest activities, this plant species was selected for further investigation, fractionation and isolation of active principles. The chemical analysis led to the isolation of five diterpenoid compounds, which were identified as coleonol B, forskolin, sugiol, 5,6-dehydrosugiol and ferruginol. It is worth to be mentioned that sugiol, 5,6-dehydrosugiol

and ferruginol are isolated for the first time from *P. barbatus* in the present study.

The *Plectranthus* diterpenoids were found to be responsible for various pharmacological activities including cytotoxic effect against several tested cancer cell lines as well as for antimicrobial activity against a lot of bacterial strains (Abdissa et al., 2017; Burmistrova et al., 2015; Yulianto et al., 2016). Our observed results for the isolated sugiol were in consistence with that obtained by Fronza et al. (2012) who reported potent cytotoxic activity and with that reported by Li et al. (2008) who reported a great antibacterial activity and with that reported by Bajpai et al. (2014) who reported strong antioxidant, lipid peroxidation inhibition and free radical scavenging efficacy. Moreover, a recent study (Xiong et al., 2017) confirmed the in this study found anticancer activity for ferruginol. It was found that ferruginol exhibited anticancer effects in OVCAR–3 human ovary cancer cells by inducing apoptosis, inhibiting cancer cell migration and inducing G2/M cell (Xiong et al., 2017). In addition to that, the in our study observed antioxidant activity was confirmed by Saijo et al. (2015) who reported a stronger antioxidant activity than the positive control carnosic acid. Furthermore, the noticed interesting antimicrobial activity of ferruginol was in agreement with literature data found confirming that ferruginol possessed a very good antimicrobial effect (Búfalo et al., 2016; Li et al., 2008). The lipophilic nature of these diterpenoid compounds could enable easy transport across the cell membranes to accumulate in and so to affect the cells.

5. Conclusion

In conclusion, the present study showed that *Plectranthus* species still represent an important resource for novel therapeutic compounds for combating cancers and infectious diseases. The investigated *Plectranthus* species possessed remarkable cytotoxic, antimicrobial and antioxidant activities. In addition, five compounds (coleonol B, forskolin, sugiol, 5,6-dehydrosugiol and ferruginol) belonging to labdane- and abietane-type diterpenes were isolated from *P. barbatus*. Interestingly, sugiol, 5,6-dehydrosuiol and ferruginol are reported for the first time from *P. barbatus* among the isolated compounds. The current results will form the basis for selection of *Plectranthus* species for further investigation in the potential discovery of new natural bioactive compounds. More investigations are needed to confirm the obtained activities and to explain the mechanism of action of the cytotoxic and antimicrobial activity. Moreover, further studies aimed at the isolation and structure elucidation of further cytotoxic, antimicrobial and antioxidative active constituents from these plant species should be continued.

Acknowledgement

The research team would like to express their gratitude for King Abdulaziz City for Science and Technology (KACST) for their financial support to the research project number (LGP-36-13). In addition, we would like to thank the Research Center at the College of Pharmacy, King Saud University for the kind support and assistance.

Conflict of interest

The authors declare that they don't have any conflict of interest.

References

Abdel Khalik, K.N., 2016. A Systematic revision of the genus *Plectranthus* L. (Lamiaceae) in Saudi Arabia based on borphological, palynological, and micromorphological characters of trichomes. Am. J. Plant Sci. 7, 1429–1444.

Abdissa, N., Frese, M., Sewald, N., 2017. Antimicrobial abietane-type diterpenoids from *Plectranthus punctatus*. Molecules 22 (11), E1919.

Aidi Wannes, W., Saidani Tounsi, M., Marzouk, B., 2017. A review of Tunisian medicinal plants with anticancer activity. J. Complement. Integr. Med. 15 (1). https://doi.org/10.1515/jcim-2017-0052.

Alasbahi, R.H., Melzig, M.F., 2010. *Plectranthus barbatus*: A review of phytochemistry, ethnobotanical uses and pharmacology – Part 1. Planta Med.. 76, 653–661.

Albadawi, D.A., Mothana, R.A., Khaled, J.M., Ashour, A.E., Kumar, A., Ahmad, S.F., Al-Said, M.S., Al-Rehaily, A.J., Almusayeib, N.M., 2017. Antimicrobial, anticancer, and antioxidant compounds from *Premna resinosa* growing in Saudi Arabia. Pharm. Biol. 55 (1), 1759–1766.

Aldulaimi, O.A., 2017. General overview of phenolics from plant to laboratory, good antibacterials or not. Pharmacogn. Rev. 11 (22), 123–127.

Alegre-Gómez, S., Sainz, P., Simões, M.F., Rijo, P., Moiteiro, C., González-Coloma, A., Martínez-Díaz, R.A., 2017. Antiparasitic activity of diterpenoids against *Trypanosoma cruzi*. Planta Med. 83 (3–04), 306–311.

Alves-Silva, J.M., Romane, A., Efferth, T., Salgueiro, L., 2017. North African medicinal plants traditionally used in cancer therapy. Front. Pharmacol. 8, 383.

Amina, M., Alam, P., Parvez, M.K., Al-Musayeib, N.M., Al-Hwaity, S.A., Al-Rashidi, N. S., Al-Dosari, M.S., 2017. Isolation and validated HPTLC analysis of four cytotoxic compounds, including a new sesquiterpene from aerial parts of *Plectranthus cylindraceus*. Nat. Prod. Res. 8, 1–6.

Bajpai, V.K., Sharma, A., Kang, S.C., Baek, K.H., 2014. Antioxidant, lipid peroxidation inhibition and free radical scavenging efficacy of a diterpenoid compound sugiol isolated from *Metasequoia glyptostroboides*. Asian Pac. J. Trop. Med. 7 (1), 9–15.

Brand, W.W., Cuvelier, H.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 82, 25–30.

Búfalo, J., Cantrell, C.L., Jacob, M.R., Schrader, K.K., Tekwani, B.L., Kustova, T.S., Ali, A., Boaro, C.S., 2016. Antimicrobial and antileishmanial activities of diterpenoids isolated from the roots of *Salvia deserta*. Planta Med. 82 (1–2), 131–137.

Burmistrova, O., Perdomo, J., Simões, M.F., Rijo, P., Quintana, J., Estévez, F., 2015. The abietane diterpenoid parvifloron D from *Plectranthus ecklonii* is a potent apoptotic inducer in human leukemia cells. Phytomedicine 22 (11), 1009–1016.

Costa, M.C.C.D., Nascimento, S.C., 2003. Actividade citotóxica de *Plectranthus barbatus* Andr. (Lamiaceae). Acta Farm Bonaerense. 22, 155–158.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564–582.

Cragg, G.M., Newman, D.J., 2000. Antineoplastic agents from natural sources: achievements and future directions. Expert Opin. Investig. Drugs. 9, 1–15.

Enioutina, E.Y., Teng, L., Fateeva, T.V., Brown, J.C.S., Job, K.M., Bortnikova, V.V., Krepkova, L.V., Gubarev, M.I., Sherwin, C.M.T., 2017. Phytotherapy as an alternative to conventional antimicrobials: combating microbial resistance. Expert Rev. Clin. Pharmacol. 10 (11), 1203–1214.

Fronza, M., Lamy, E., Günther, S., Heinzmann, B., Laufer, S., Merfort, I., 2012. Abietane diterpenes induce cytotoxic effects in human pancreatic cancer cell line MIA PaCa-2 through different modes of action. Phytochemistry 78, 107–119.

Gaspar-Marques, C., Rijo, P., Simões, M.F., Duarte, M.A., Rodriguez, B., 2006. Abietanes from *Plectranthus grandidentatus* and *Plectranthus hereroensis* against methicillin and vancomycin-resistant bacteria. Phytomedicine 13, 267–271.

Hirasawa, Y., Izawa, E., Matsuno, Y., Kawahara, N., Goda, Y., Morita, H., 2007. Taxodistines A and B, abietane-type diterpenes from *Taxodium distichum*. Bioorg. Med. Chem. Lett. 17 (21), 5868–5871.

Joshi, R.K., 2014. Chemical composition and antimicrobial activity of the essential oil of *Plectranthus mollis* (Lamiaceae) from Western Ghats Region, Karnataka, India. Rev. Biol. Trop. 62 (2), 423–431.

Kisangau, D.P., Hosea, K.M., Joseph, C.C., Lyaruu, H.V., 2007. *In vitro* antimicrobial assay of plants used in traditional medicine in Bukoba Rural district, Tanzania. Afr. J. Tradit. Complement Altern. Med. 4 (4), 510–523.

Kolak, U., Topcu, G., Birteksoz, S., Otuk, G., Ulubelen, A., 2005. Terpenoids and steroids from the roots of *Salvia blepharochlaena*. Turk. J. Chem. 29 (2), 177–186.

Kusumoto, N., Ashitani, T., Murayama, T., Ogiyama, K., Takahashi, K., 2010. Antifungal abietane-type diterpenes from the cones of *Taxodium distichum* Rich. J. Chem. Ecol. 36 (12), 1381–1386.

Li, W.H., Chang, S.T., Chang, S.C., Chang, H.T., 2008. Isolation of antibacterial diterpenoids from *Cryptomeria japonica* bark. Nat. Prod. Res. 22 (12), 1085–1093.

Li, M., Zhang, J.S., Ye, Y.M., Fang, J.N., 2000. Constituents of the roots of *Salvia prionitis*. J. Nat. Prod. 63 (1), 139–141.

Luis, J.G., Grillo, T.A., 1993. New diterpenes from *Salvia munzii*: chemical and biogenetic aspects. Tetrahedron 49 (28), 6277–6284.

Lukhoba, C.W., Simmonds, M.S.J., Paton, A.J., 2006. *Plectranthus*: a review of ethnobotanical uses. J. Ethnopharmacol. 103, 1–24.

Mann, C.M., Markham, J.L., 1998. A new method for determining the minimum inhibitory concentration of essential oils. J. Appl. Microbiol. 84, 538–544.

Mbaveng, A.T., Kuete, V., Efferth, T., 2017. Potential of central, eastern and western Africa medicinal plants for cancer therapy: spotlight on resistant cells and molecular targets. Front. Pharmacol. 8, 343.

Mohd-Esa, N., Hern, F.S., Ismail, A., Yee, C.L., 2010. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chem. 122, 1055–1060.

Mothana, R.A.A., Grünert, R., Lindequist, U., Bednarski, P.J., 2007. Study of the anticancer potential of Yemeni plants used in folk medicine. Pharmazie 62, 305–307.

Mothana, R.A., Al-Said, M.S., Al-Musayeib, N.M., El Gamal, A.A., Al-Massarani, S.M., Al-Rehaily, A.J., Abdulkader, M., Maes, L., 2014. In vitro antiprotozoal activity of abietane diterpenoids isolated from *Plectranthus barbatus*. Andr. Int. J. Mol. Sci. 15 (5), 8360–8371.

Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981–2002. J. Nat. Prod. 66, 1022–1037.

Ngezahayo, J., Havyarimana, F., Hari, L., Stévigny, C., Duez, P., 2015. Medicinal plants used by Burundian traditional healers for the treatment of microbial diseases. J. Ethnopharmacol. 173, 338–351.

Okeke, I.N., Laxmaninarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A., Klugman, K.P., 2005. Antimicrobial resistance in developing countries. Part1: recent trends and current status. Lancet Infect Dis. 5, 481–493.

Orabi, K.Y., Mossa, J.S., Muhammed, I., Alloush, M.H., Galal, A.M., El-Feraly, F.S., McPhail, A.T., 2000. New eudesmane sesquiterpenes from *Plectranthus cylindraceus*. J. Nat. Prod. 63 (12), 1665–1668.

Prakash, O., Roy, R., Tandon, J.S., Dhar, M.M., 1988. Carbon-13 and proton two-dimensional NMR study of diterpenoids of *Coleus forskohlii*. Magn. Reson. Chem. 26 (2), 117–119.

Russell, G.B., 1975. Lignans and sugiol from *Libocedrus bidwillii*. Phytochemistry 14 (12). 2708–2708.

Saeed, M.E.M., Meyer, M., Hussein, A., Efferth, T., 2016. Cytotoxicity of South-African medicinal plants towards sensitive and multidrug-resistant cancer cells. J. Ethnopharmacol. 186, 209–223.

Saijo, H., Kofujita, H., Takahashi, K., Ashitani, T., 2015. Antioxidant activity and mechanism of the abietane-type diterpene ferruginol. Nat. Prod. Res. 29 (18), 1739–1743.

Saksena, A.K., Green, M.J., Shue, H.j., Wong, J.K., McPhail, A.T., Gross, P.M., 1985. Identity of coleonol with forskolin: structure revision of a base-catalysed rearrangement product. Tetrahedron Lett. 26 (5), 551–554.

Stavri, M., Paton, A.J., Skelton, B.W., Gibbons, S., 2009. Antibacterial diterpenes from *Plectranthus ernstii*. J. Nat. Prod. 72, 1191–1194.

Sultana, S., Asif, H.M., Nazar, H.M., Akhtar, N., Rehman, J.U., Rehman, R.U., 2014. Medicinal plants combating against cancer–a green anticancer approach. Asian Pac. J. Cancer. Prev. 15 (11), 4385–4394.

Van Zyl, R.L., Khan, F., Edwards, T.J., Drewes, S.E., 2008. Antiplasmodial activities of some abietane diterpenes from the leaves of five *Plectranthus* species. S. Afr. J. Sci. 104, 62–64.

Viswanathaswamy, A.H., Koti, B.C., Gore, A., Thippeswamy, A.H., Kulkarni, R.V., 2011. Antihyperglycemic and antihyperlipidemic activity of *Plectranthus amboinicus* on normal and alloxan-induced diabetic rats. Indian J. Pharm. Sci. 73 (2), 139–145.

Wang, C.Y., Bai, X.Y., Wang, C.H., 2014. Traditional Chinese medicine: a treasured natural resource of anticancer drug research and development. Am. J. Chin. Med. 42 (3), 543–559.

World Health Organization, 2014. Attaining the nine global noncommunicable diseases targets; a shared responsibility. Geneva: WHO, 2014. Global Status Report on noncommunicable diseases.

World Health Organization, 2017. Cancer. Fact sheet.

Wong, J.H., Cho Wing Sze, S., Ng, T.B., Chi Fai Cheung, R., Tam, C., Zhang, K.Y., Dan, X., et al., 2017. Apoptosis and anti-cancer drug discovery: the power of medicinal fungi and plants. Curr. Med. Chem. https://doi.org/10.2174/0929867324666170720165005.

Xiong, W.D., Gong, J., Xing, C., 2017. Ferruginol exhibits anticancer effects in OVCAR–3 human ovary cancer cells by inducing apoptosis, inhibition of cancer cell migration and G2/M phase cell cycle arrest. Mol. Med. Rep. 16 (5), 7013–7017.

Ying, B.-P., Kubo, I., 1991. Complete ^{1}H and ^{13}C NMR assignments of totarol and its derivatives. Phytochemistry 30 (6), 1951–1955.

Yoshikawa, K., Tanaka, T., Umeyama, A., Arihara, S., 2006. Three abietane diterpenes and two diterpenes incorporated sesquiterpenes from the bark of *Cryptomeria japonica*. Chem. Pharm. Bull. (Tokyo) 54 (3), 315–319.

Yulianto, W., Andarwulan, N., Giriwono, P.E., Pamungkas, J., 2016. HPLC-based metabolomics to identify cytotoxic compounds from *Plectranthus amboinicus* (Lour.) Spreng against human breast cancer MCF-7Cells. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1039, 28–34.