Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Flavonoidal Constituents, Antioxidant, Antimicrobial, and Cytotoxic Activities of *Dipterygium glaucum* Grown in Kingdom of Saudi Arabia

Usama Shaheen[1,2], Nagwa Abdelkader Shoeib[1,3], Abeer Temraz[1,2], Mohamed I. S. Abdelhady[1,4]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah Al Mukarramah 21955, Saudi Arabia, [2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, [3]Department of Pharmacognosy, Faculty of Pharmacy, Tanta University, Tanta 31527, [4]Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt

Submitted: 21-01-2016 Revised: 11-07-2016 Published: 11-10-2017

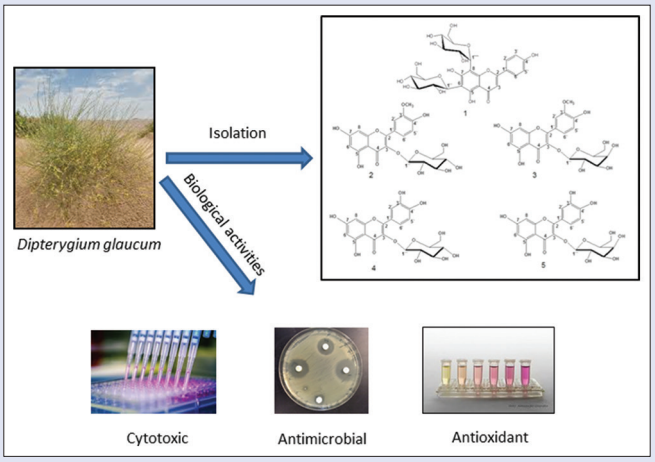
ABSTRACT

Background: *Dipterygium glaucum* Decne. herb is one of the common traditional plants with multiple medicinal uses. **Objective:** To isolate the major constituents and to investigate the antioxidant, antimicrobial, and cytotoxic activities of this herb. **Materials and Methods:** Methanolic extract of *D. glaucum* herb was fractionated using *n*-hexane, dichloromethane, and *n*-butanol. Butanol fraction was chromatographed using column chromatography and preparative thin layer chromatography to isolate the major constituents. Isolated compounds were elucidated by means of spectroscopic methods, including 1D, 2D NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC, NEOSY) and MS analysis. Total phenolic content using Folin–Ciocalteu reagent and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay of the total methanolic extract were evaluated. Cytotoxic potential of both methanolic extract and butanol fraction was tested using a crystal violet viability assay. Antimicrobial activities of both extracts were investigated using diffusion agar technique. **Results:** Apigenin 6, 8-di-C-glucopyranoside (vicenin-2), quercetin-3`-*O*-methyl-3-*O*-glucopyranoside, quercetin-3`-*O*-methyl-3-*O*-galactopyranoside, quercetin-3-*O*-*β*-D-glucopyranoside, and quercetin-3-*O*-*β*-D-galactopyranoside were isolated and elucidated. Total phenolic content was (83.89 mg gallic acid equivalent/g extract). The EC_{50} value of scavenging DPPH radical was 152.0 ± 2 μg/mL. Butanol fraction showed the highest cytotoxic activity against cervical and breast carcinoma cells (IC_{50} 3.6 and 6.1 μg/mL, respectively). Both methanolic extract and butanol fraction showed wide spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria and some fungi. The highest activity was from methanolic extract against *Enterococcus faecalis* (83.25%) and against *Candida tropicalis* (77.03%) as compared to reference antibiotics. **Conclusion:** Data obtained from this study demonstrate that *D. glaucum* possesses significant antioxidant, cytotoxic, and antimicrobial activities which could be ascribed to its flavonoidal content.

Key words: Antimicrobial, antioxidant, cytotoxic, *Dipterygium glaucum*, flavonoids

SUMMARY

- *Dipterygium glaucum* Decne. herb is one of the common traditional plants with multiple medicinal uses in KSA
- Five flvonidal glycosides were isolated and elucidated
- This study demonstrated that *D. glaucum* possesses significant antioxidant, cytotoxic, and antimicrobial activities.



Abbreviations used: KSA: Kingdom of Saudi Arabia; TLC: Thin Layer Chromatography; DPPH: 2,2`-diphenyl-1-picrylhydrazyl; EC50: Half maximal effective concentration; IC_{50}: Half maximal inhibitory concentration; DMSO: dimethyl sulfoxide; NMR: Nuclear Magnetic Resonance; ESIMS: Electrospray ionization mass spectrometry; MeOH: Methyl alcohol.

Correspondence:

Dr. Nagwa Abdelkader Shoeib,
Department of Pharmacognosy,
Faculy of Pharmacy, Umm Al-Qura University,
Makkah Al Mukarramah 21955,
Saudi Arabia.
E-mail: nashoeib@uqu.edu.sa; na2001eg@yahoo.com

DOI: 10.4103/pm.pm_44_16

Access this article online
Website: www.phcog.com
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INTRODUCTION

The use of medicinal plants as an essential component for traditional healthcare systems is the oldest and the most varied of all therapeutic systems. The World Health Organization estimated that 80% of the world population currently uses herbal medicine for some aspects of primary health care.[1]

In Saudi Arabia, traditional medicine is based on herbal remedies and spiritual healing. It is not possible to find a city or village where traditional herbal medicines are not used.[2]

Cite this article as: Shaheen U, Shoeib NA, Temraz A, Abdelhady MI. Flavonoidal constituents, antioxidant, antimicrobial, and cytotoxic activities of *Dipterygium glaucum* grown in Kingdom of Saudi Arabia. Phcog Mag 2017;13:S484-8.

Dipterygium glaucum Decne. herb (*Capparidaceae*) is a branched under shrub plant. It is a monotypic genus with one species, widely distributed in Saudi Arabia, Egypt, Sudan, and Pakistan.[3,4]

It is one of the common plants that are used by the Bedouins in Saudi Arabia. It has multiple medicinal uses; it is popular for the treatment of miss-breathing troubles as trachea dilating agent.[5]

It is also used traditionally in Pakistan as decoction and infusion for the treatment of jaundice, blood purifier, psoriasis, and ringworm infestation and as an antiasthma drug.[4,6]

Phytochemical studies on *D. glaucum* revealed the presence of alkaloids, cardiac glycoside, bound anthraquinones, saponins, terpenoids, and sterols.[3,7]

Previous biological studies on the plant revealed that ethanolic plant extract has antioxidant and antispasmodic activities while *n*-hexane, ethyl acetate, and butanol extracts have phytotoxic activity.[8]

Oxidative stress is the leading cause for the development of many recent diseases such as arteriosclerosis, inflammation, autoimmune diseases, cancer, and aging. Antioxidants deactivate and stabilize reactive oxygen species and other oxidants before they affect biological cells.[9]

Food and herbal plants contain many classes of antioxidant constituents such as flavonoids, phenolics, proanthocyanidins, and tannins. These constituents have free radical scavenging abilities and contribute to the protection of human body from diseases.[10]

Microbial resistance is considered serious threat to public health as infections caused by resistant microorganisms often fail to respond to the standard treatment. Natural products, in the form of pure compounds or standardized extracts, offer excellent chances for new drug discovery due to the chemical diversity of naturally derived compounds.[11]

As phytochemicals are important compounds that play an essential role for restoring normal functioning of human body and amelioration of diseases, this study was designed to isolate the major phytochemical constituents and to investigate the antioxidant, antimicrobial, and cytotoxic activities of the wild *D. glaucum* collected from Makkah deserts, Kingdom of Saudi Arabia (KSA).

MATERIALS AND METHODS

Plant material

The aerial parts of *D. glaucum* herb were collected in February 2013 from Makkah, KSA, and was identified by Mohamed Meelad Late, Professor of Plant Taxonomy, Faculty of Applied Sciences, Umm Al-Qura University. A voucher specimen (DG 013) is deposited at Department of Pharmacognosy, College of Pharmacy, Umm Al-Qura University.

Extraction and isolation

The dried powdered aerial parts of *D. glaucum* (1.5 kg) were exhaustively extracted with methanol by maceration at room temperature (3 × 6 L). Methanolic extract was evaporated under reduced pressure using rotary evaporator (Buchi Co., Switzerland) till dryness to give 368.5 g dry extract. Dry extract (200 g) was suspended in distilled water (350 mL) and was fractionated using *n*-hexane, dichloromethane, and *n*-butanol (3 × 500 mL). The fractions were collected and evaporated till dryness to give the following weights, 25 g, 12 g, and 78 g. Butanol fraction (78 g) was chromatographed on silica gel column (Sigma-Aldrich, USA) (5 cm × 120 cm, flow rate 2.5 mL/min) and was eluted by chloroform/methanol mixtures of increasing polarities by the addition of methanol in 10% increments till 100% methanol. Fractions of 50 mL were collected, monitored by thin layer chromatography (TLC) (aluminum-coated silica gel 60 F_{254}, Macherey-Nagel, Germany) using chloroform-methanol-water (80:20:2, v/v/v) and chloroform-methanol-water-acetic acid (60:32:7:1 v/v/v/v) as mobile phases, and spots were detected on the plates using methanol/sulfuric acid (20% v/v) spray reagent; then, similar fractions were grouped into 18 major fractions (A-R).

Fraction L (3.99 g) was chromatographed on Sephadex LH-20 column (Pharmacia, Uppsala, Sweden) (2.5 cm × 30 cm, flow rate 1.0 mL/min) using methanol as the eluent to give nine fractions. Fraction 3 was rechromatographed on Sephadex LH-20 column (1.5 cm × 20 cm, flow rate 0.5 mL/min) using methanol as the eluent to give three subfractions. Subfraction 1 was subjected to silica gel column (1.0 cm × 15 cm, flow rate 0.5 mL/min) using chloroform-methanol-water (8:20:2 v/v/v) as starting eluent, then increasing the polarity to 70:30:3 v/v/v which affords compounds 4 and 5.

Fraction M (230 mg) was chromatographed on Sephadex LH-20 column (2.5 cm × 30 cm, flow rate 1.0 mL/min) using methanol as eluent, which gave four subfractions. Subfraction number 4 was subjected to preparative TLC using chloroform-methanol-water-acetic acid (60:32:7:1 v/v/v/v), and bands at R_f 0.5 and 0.55 were scratched, extracted with methanol, and purified using small Sephadex column; this affords compounds 2 and 3.

Fraction R (600 mg) was applied to Sephadex LH-20 column (2.5 cm × 30 cm, flow rate 1.0 mL/min) and was eluted using methanol, this afforded six subfractions. Subfraction number 5 was separated by silica gel column (1.5 cm × 15 cm, flow rate 0.5 mL/min) using ethyl acetate-methanol mixture (80:20 v/v) as eluent, which gave seven fractions. Fraction number 5 was subjected to preparative TLC using chloroform-methanol-water-acetic acid (60:32:7:1 v/v/v/v) as mobile phase, band at R_f 0.3 was scratched, extracted with methanol, and purified using sephadex column to give compound 1.

Compound 1 (6,8-Di-C-glucosylapigenin or vicenin-2)

Yellow amorphous powder; UV λmax MeOH 232, 282, 344 nm. ESI MS (m/z): 593 [M-H]-, 503, 473, 383, and 353. ^{1}H nuclear magnetic resonance (NMR) (500 MHz, dimethyl sulfoxide [DMSO]-d6) δ: 8.03 (2H, d, *J* = 8.4 Hz, H-2′, 6′), 6.93 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 6.82 (^{1}H, s, H-3); 6-C-β-Glc: 4.81 (^{1}H, d, *J* = 9.7 Hz, H-1′′); 8-C-β-Glc:

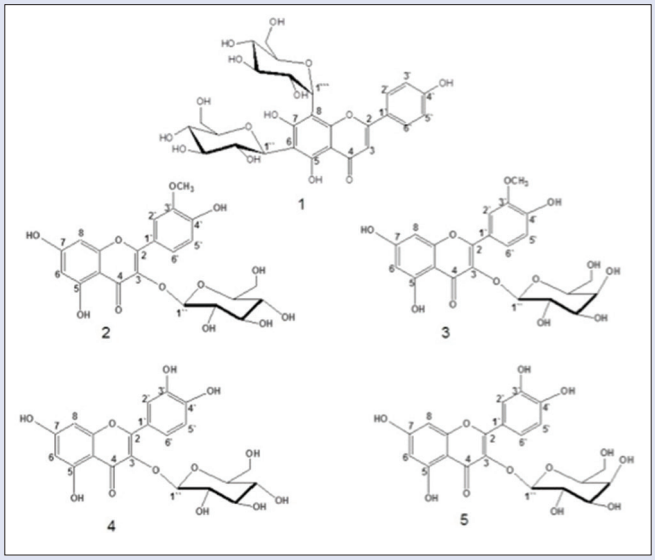

Figure 1: Structure of compounds 1-5 isolated from *Dipterygium glaucum*

4.76 (^{1}H, d, J = 9.8 Hz, H-1′′′). ^{13}C NMR (125MHz, DMSO-d6) δ_c 182.8 (C-4), 164.5 (C-2), 161.7 (C-3), 161.69 (C-4`), 159.0 (C-5), 155.5 (C-9), 129.5 (C-6`),129.5 (C-2`), 122.0 (C-1`), 116.3 (C-5`), 116.3 (C-3`), 107.9 (C-6), 105.7 (C-10), 104.3 (C-8), 103.0 (C-3), 82.3 (C-5``), 82.3 (C-5```), 79.3 (C-3``), 78.3 (C-3```), 74.5 (C-1``), 73.8 (C-1```), 72.4 (C-2``), 71.4 (C-2```), 71.0 (C-4``), 69.5 (C-4```), 61.7 (C-6``), 60.2 (C-6```).

Compound 2 (quercetin-3`-*O*-methyl-3-*O*-glucopyranoside)

It was obtained as a light yellow amorphous powder; UV λ_{max} MeOH: 258, 305, 361 nm. ESI-MS: m/z 479.0 [M + H]$^+$. ^{1}H NMR (500 MHz, DMSO-d6) δ: 7.55 (^{1}H, brs, H-2′), 7.50 (^{1}H, d, J = 8.4 Hz, H-6′), 6.70 (^{1}H, d, J = 7.9 Hz, H-5′), 6.37 (^{1}H, d, J = 1.8 Hz, H-8), 6.17 (^{1}H, d, J = 1.8 Hz, H-6), 3.80 (3H, s, OCH_3-3'); 3-O-β-Glc: 5.32 (^{1}H, d, J = 7.3 Hz, H-1′′). ^{13}C NMR (125 MHz, DMSO-d6) δ_c 175.2 (C-4), 160.1 (C-5), 156.2 (C-2), 156.2 (C-9), 144.9 (C-3`),133.1 (C-3), 121.8 (C-1`), 121.8 (C-6`), 116.4 (C-2`), 116.2 (C-5`), 103.3 (C-10), 101.5 (C-1``), 99.9 (C-6), 94.9 (C-8), 77.8 (C-3``), 77.1 (C-5``), 74.6 (C-2``), 70.4 (C-4``), 61.4 (C-6``), 56.03(OCH3).

Compound 3 (quercetin-3`-*O*-methyl-3-*O*-galactopyranoside)

Yellow powder, UV λ_{max} MeOH: 353 and 254 nm; ESI-MS: m/z 477.0 [M$^-$ H]$^-$. ^{1}H NMR (500 MHz, DMSO-d6) δ: 7.54 (^{1}H, brs, H-2′), 7.53 (^{1}H, d, J = 8.4 Hz, H-6′), 6.73 (^{1}H, d, J = 8.4 Hz, H-5′), 6.04 (^{1}H, d, J = 2 Hz, H-8), 5.86 (^{1}H, d, J = 2 Hz, H-6), 3.82 (3H, s, OCH_3-3'); 3-O-β-Gla: 5.22 (^{1}H, d, J = 7.8 Hz, H-1′′). ^{13}C NMR (125MHz, DMSO-d6) δ_c 176.3 (C-4), 161.2 (C-5), 157.3 (C-2), 157.3 (C-9), 146.0 (C-3`), 133.3 (C-3), 121.8 (C-1`), 121.8 (C-6`), 115.9 (C-2`), 115.8 (C-5`), 103.3 (C-10), 102.3 (C-1``), 99.9 (C-6), 94.9 (C-8), 74.0 (C-3``), 76.2 (C-5``), 71.7 (C-2``), 68.3 (C-4``), 60.5 (C-6``), 56.26 (OCH3).

Compound 4 (quercetin 3-*O*-*β*-D-glucopyranoside)

Yellow amorphous powder; EI-MS m/z 463.08 [M$^-$ H]$^-$; ^{1}H-NMR (DMSO-d6, 500 MHz) δ: 7.54 (^{1}H, d, J = 1.3, H-2′), 7.65 (^{1}H, dd, J = 6.8, 1.3 Hz, H-6′), 6.80 (^{1}H, d, J = 6.8 Hz, H-5′), 6.37 (^{1}H, d, J = 2 Hz, H-8), 6.17 (^{1}H, d, J = 2 Hz, H-6); 3-O-β-Glc: 5.45 (^{1}H, d, J = 7.5 Hz, H-1′′). ^{13}C NMR (125 MHz, DMSO-d6) δ_c 177.7 (C-4), 165.9 (C-7), 161.6 (C-5), 156.5 (C-2), 156.8 (C-9),149.1 (C-4`), 145.4 (C-3`), 133.8 (C-3), 121.4 (C-1`), 122.3 (C-6`), 116.4 (C-2`), 115.6 (C-5`), 104.0 (C-10), 101.4 (C-1``), 99.4 (C-6), 94.1 (C-8), 77.9 (C-3``), 77.0 (C-5``), 74.5 (C-2``), 70.4 (C-4``), 61.4 (C-6``).

Compound 5 (quercetin-3-*O*-*β*-D-galactopyranoside)

Yellow powder, UV λ_{max} MeOH: 353 and 254 nm; EI-MS m/z 463.07 [M$^-$ H]$^-$; ^{1}H-NMR (DMSO-d6, 500 MHz) δ: 7.56 (^{1}H, brs, H-2′),7.65 (^{1}H, d, J = 8.5 Hz, H-6′), 6.81 (^{1}H, d, J = 8.5 Hz, H-5′), 6.38 (^{1}H, d, J = 1.71 Hz, H-8), 6.17 (^{1}H, d, J = 1.71 Hz, H-6); 3-O-β-Gla: 5.36 (^{1}H, d, J = 7.5 Hz, H-1′′). ^{13}C NMR (125 MHz, DMSO-d6) δ_c 177.7 (C-4), 165.9 (C-7), 161.6 (C-5), 156.5 (C-2), 156.8 (C-9), 149.1 (C-4`), 145.3 (C-3`), 133.8 (C-3), 121.4 (C-1`), 122.3 (C-6`), 116.3 (C-2`), 115.6 (C-5`), 103.9 (C-10), 102.4 (C-1``), 99.4 (C-6), 94.1 (C-8), 73.6 (C-3``), 76.2 (C-5``), 71.6 (C-2``), 68.3 (C-4``), 60.5 (C-6``) [Figure 1].

Quantitative estimation of total phenolic content

Total phenolic content in the plant methanolic extract was determined by Folin–Ciocalteu method.[12] Calibration curve was constructed as follows: 1 mL of 30, 60, 90, 120, 150, and 180 µg/mL ethanolic gallic acid solutions was added to 5 mL Folin–Ciocalteu reagent (diluted 10-fold) and 4 mL sodium carbonate (75 g/L). The absorbance was measured after 30 min at 765 nm using a spectrophotometer (UV2550, Shimadzu, Japan) and the calibration curve was plotted. 0.2 mL methanolic extract (1 mg/mL) was mixed with the same reagents as described above, and the absorbance was measured. All determinations were done in triplicate.

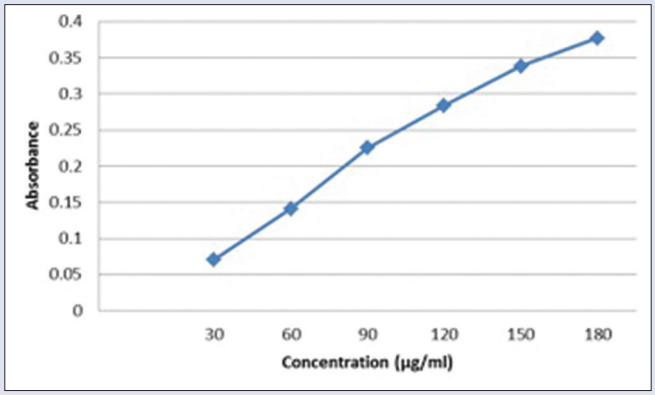

Figure 2: Standard calibration curve of gallic acid

The total phenolic content was calculated using the following linear equation based on the calibration curve of gallic acid [Figure 2].

$A = 0.008X - 0.019$.

where A is the absorbance and X is the amount of gallic acid.

The result was expressed as gallic acid equivalent (GAE)/g.

In vitro determination of antioxidant activity

In vitro free radical scavenging activity of the methanolic extract was measured using 2,2`-diphenyl-1-picrylhydrazyl (DPPH) assay according to the published method.[13] Extract solution was prepared by dissolving 10 mg of dry extract in 10 mL methanol. The solution of DPPH (Sigma-Aldrich, KSA) in methanol (6×10^{-5} M) was freshly prepared daily. Three milliliters of DPPH solution was mixed with 20, 40, 60, 80, 100, and150 µl extract solution in 1 cm path length microcuvettes. The samples were kept in the dark at room temperature for 15 min, and then, the decrease in the absorbance was measured using the spectrophotometer. Blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

% inhibition = [(AB − AA)/AB] × 100

where AB is the absorbance of DPPH (t = 0 min) and AA is the absorbance of plant extract solution + DPPH (t = 15 min).

In vitro cytotoxic activity against human cell lines

The cytotoxicity of methanolic extract and butanol fraction against hepatocellular (HepG2), breast (MCF-7), pharynx (HEP-2), colon (HCT), and cervical (HeLa) carcinoma human cell lines was tested by a crystal violet viability assay,[14,15] and IC_{50} values are determined and illustrated in Table 1.

Antimicrobial activity

Diffusion agar technique was applied using trypticase soy agar (Difco) medium containing bacterial or fungal suspension of the test organisms.[16] Impregnated discs with plant extracts, DMSO as negative control and amphotericin B, ampicillin, gentamicin as standard

antibiotics, were placed on the surface of the cultured agar and were incubated at 35°C–37°C for 24–48 h in case of bacteria and at 25°C for 48 h in case of fungi. After incubation, zones of inhibition diameters were measured in millimeters and the results are shown in Table 2.

RESULTS

Phytochemical investigation of *D. glaucum* led to the isolation of one *C*-flavone glycoside (compound 1) and four *O*-flavonol glycosides, compounds (2-5). They were elucidated by means of spectroscopic methods including 1D, 2D NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC), MS analysis and by the comparison of the physical and spectral data with those published before as apigenin 6, 8-di-C-glucopyranoside (vicenin-2),[17] quercetin-3`-*O*-methyl-3-*O*-glucopyranoside,[18] quercetin-3`-*O*-methyl-3-*O*-galactopyranoside,[19] quercetin-3-*O*-*β*-D-glucopyranoside,[20] quercetin-3-*O*-*β*-D-galactopyranoside[21] [Figure 1].

Quantitative estimation of total phenolic content

Phenolic content of the methanolic extract of the studied plant was 83.89 mg/g expressed as GAEs.

Antioxidant activity

Methanolic extract of *D. glaucum* showed concentration-dependent scavenging effect on DPPH radical [Figure 3]. The EC_{50} value of scavenging DPPH radical was found to be 152.0 ± 2 µg/mL.

Cytotoxic activity

Methanolic extract and butanol fraction of *D. glaucum* exhibited cytotoxic activity against tested cell lines. Butanol fraction showed the highest cytotoxic activity against cervical and breast carcinoma cells (IC_{50} 3.6 and 6.1 µg/mL, respectively).

Antimicrobial activity

Methanolic extract and butanol fraction of *D. glaucum* showed comparable antibacterial activity against Gram-positive bacteria; the highest activity was obvious from methanolic extract against *Enterococcus faecalis* (83.25%), while both extracts showed weak activity against tested Gram-negative bacteria. Both extracts showed moderate antifungal activity. Methanolic extract showed the highest activity against *Candida tropicalis* (77.03%) [Table 2].

Table 1: Results of cytotoxicity of methanolic extract and butanol fraction of *Dipterygium glaucum*

Extract	IC_{50} (µg/ml)				
	HCT	HEP-2	HeLa	HepG2	MCF-7
Butanol fraction	35.7	24.6	3.6	22.3	6.1
Methanolic extract	18.6	14.4	24.7	28.3	35.2

DISCUSSION

This study reported the isolation of *C*-and *O*-flavonoidal glycosides from *D. glaucum* for the first time.

During the present investigation of *D. glaucum*, the methanolic extract showed concentration-dependent antioxidant activity against DPPH radical by colored solution bleaching with EC_{50} 152.0 ± 2 µg/mL. This inhibition percentage could be correlated positively to phenolic content of the plant.

D. glaucum butanol fraction demonstrated a promising anticancer activity against cervical (HeLa) and breast (MCF-7) carcinoma cells, and this could be attributed to flavonoidal content of the plants.

Flavonoids are naturally occurring plants secondary metabolites; they have positive impact on human health. They are highly effective scavengers against most oxidizing molecules as free radicals and singlet oxygen species are responsible for many diseases as cancer.[22]

Previous study was conducted to determine synergistic effect of vicenin-2 in combination with docetaxel in prostate cancer treatment. The authors reported that vicenin-2 is effective against

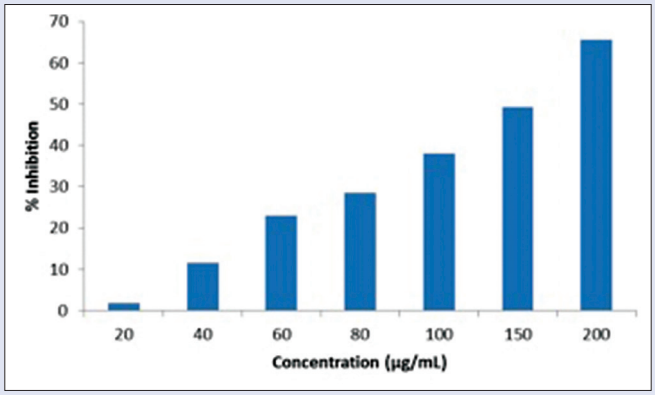

Figure 3: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of *Dipterygium glaucum* methanolic extract

Table 2: Antimicrobial activities of methanolic extract and butanol fraction of *Dipterygium glaucum*

Tested organism	Sample		
Gram-positive	**Butanol fraction (%)**	**Methanolic extract (%)**	**Standard ampicillin**
Streptococcus pneumonia (RCB 010010)	14.8±0.34 (62.18)	15.3±0.34 (64.28)	23.8±0.2
Enterococcus faecalis (RCMB 010068)	16.4±0.19 (78.84)	16.9±0.62 (83.25)	20.3±0.3
Staphylococcus aureus (RCMB 010028)	13.2±0.67 (46.64)	15.4±0.38 (54.42)	28.3±0.1
Gram-negative	**Butanol fraction (%)**	**Methanolic extract (%)**	**Standard gentamicin**
Escherichia coli (RCMB 010052)	9.6±0.34 (47.05)	11.4±0.44 (55.88)	20.4±0.6
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	18.2±0.1
Fungi	**Butanol fraction (%)**	**Methanolic extract (%)**	**Standard amphotericin B**
Aspergillus fumigatus (RCMB 02568)	12.6±0.23 (53.16)	14.5±0.35 (61.18)	23.7±0.1
Candida tropicalis (RCMB 05239)	15.8±0.51 (75.59)	16.1±0.38 (77.03)	20.9±0.3
Cryptococcus neoformans (RCMB 05642)	14.1±0.55 (64.38)	16.2±0.46 (73.97)	21.9±0.12

Results are expressed by mean zone of inhibition (in mm)±SD (potency %). RCMB: Regional Center for Mycology and Biotechnology; NA: No activity; SD: Standard deviation

prostate carcinoma progression when coadministered with docetaxel than either of the single agents in androgen-independent prostate cancer.[23]

Isorhamnetin is found in many plants and is a metabolite of quercetin. Lee *et al*.[24] demonstrated the anticancer effect of isorhamnetin on lung cancer cell culture and on mice diseased with lung cancer. *In vitro* study confirmed that isorhamnetin induces apoptosis, which is mediated by mitochondria-dependent caspase activation. *In vivo* test proved the anticancer effect of isorhamnetin; weights of tumors significantly reduced by injecting mice with isorhamnetin.[24]

In this study, the isolation of vicenin-2 (compound 1) together with isorhamnetin and quercetin glycosides (compounds 2-5) from butanol fraction suggested that flavonoids could be the major group of constituents that may be responsible for the anticancer activity.

Diseases resulting from microbial infection represent an important cause of morbidity and mortality among human and animals, particularly in developing countries. Therefore, searching for new antimicrobial drugs from medicinal plants has greatly increased in recent years, especially due to the constant rise of microorganisms resistant to conventional medications.

According to Alves *et al*.,[25] inhibition zones > 9 mm correspond to active antimicrobial samples. In the present study, *D. glaucum* methanolic extract and butanol fraction showed wide spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. The highest activity was from methanolic extract against *E. faecalis* (83.25%) and against *C. tropicalis* (77.03%) as compared to reference antibiotics.

In previous work,[3] it was reported that *D. glaucum* has no antimicrobial activity, and in the same study, they reported the absence of flavonoids in the plant extract. Herein, we report the isolation of flavonoidal glycosides which may be the responsible constituents for the reported activity and the slight increase in the activity of methanolic extract over butanol could be attributed to synergistic activity of flavonoidal glycosides with other secondary metabolites in the plant extract.

CONCLUSION

The present finding showed that *D. glaucum* has antimicrobial, cytotoxic, and antioxidant activities, thus demonstrating its potential benefits in ameliorating many diseases. Further studies are required to identify the mechanisms, whereby *D. glaucum* exerts its beneficial effects.

Financial support and sponsorship

The authors would like to thank the Deanship of Scientific Research and the Institute of Scientific Research and Revival of Islamic Heritage at Umm Al-Qura University (Project ID: 4331009) for the financial support.

Conflicts of interest

There are no conflicts of interest.

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