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Chemical composition, antioxidant, antimicrobial, and anticancer activities of *Mahonia napaulensis* DC. bark from Nepal

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Abstract

Background Cancer is one of the major health problems worldwide and medicinal plants constitute a common alternative for cancer treatment having no or fewer side effects. This study aimed to assess total phenolic (TPC) and flavonoid (TFC) contents, antioxidant, biological activities (especially antibacterial, antifungal, and anticancer), and chemical composition of methanol extract of *M. napaulensis* DC. bark (MNBM). This is the first study evaluating its anticancer activity and chemical composition by LC–MS/MS analysis.

Methods TPC, TFC, antioxidant, antimicrobial, and anticancer activities were determined by Folin-Ciocalteu, $AlCl_3$, DPPH, Resazurin, and MTT assays, respectively. Its metabolite profiling was done by LC–MS/MS analysis. The statistical significance of differences between test groups was analyzed by a one-way ANOVA.

Results The preliminary phytochemical screening revealed the presence of various phytochemicals viz. alkaloids, steroids, glycosides, polyphenols, tannins, flavonoids, coumarins, terpenoids, and quinone. MNBM showed 38.00 ± 1.50 mg GAE g^{-1} dry sample as TPC, 35.04 ± 4.87 mg QE g^{-1} dry sample as TFC, and 212.97 µg/mL IC_{50} value ($P < 0.05$) as moderate antioxidant activity. MNBM showed minimal inhibitory concentration (MIC) values of 100.22 mg/mL, 50.15 mg/mL, and 25.08 mg/mL against *S. aureus, E. coli*, and *C. albicans,* respectively as weak antimicrobial activity. It showed no antibacterial effect against *B. cereus* and *P. aeruginosa* at 120 mg/mL. The anticancer activity of MNBM was moderate against human lung cancer cells A549 (228.97 µg/mL IC_{50} value) and human cervical cancer cells HeLa (367.72 µg/mL IC_{50} value) ($P < 0.05$). The LC–MS/MS analysis reported the presence of different anticancer compounds viz. dihydroberberine, d-berbamine, (S)-glaucine, protopine, grosheimin, mycophenolic acid, berberine, alpha-linolenic acid, etc.

Conclusions MNBM showed dose-dependent moderate antioxidant, weak antibacterial, weak antifungal, and moderate anticancer activity due to the synergistic effect of different phytochemicals and anticancer compounds.

Keywords *Mahonia napaulensis* DC., Antioxidant, Antimicrobial, Anticancer, Phytochemicals, DPPH, Resazurin, MTT, LC–MS/MS

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Background

Ayurveda is a 5000 years old traditional medical system that originated and developed in the Indian sub-continent. Ayurveda literally means *"science of long life"* as it is made up of two Sanskrit words, *Ayus* (long life) and *Veda* (knowledge or science) [1]. According to the World Health Organization (WHO), about 65% of the world's population and 80% of developing countries' population depend on about 85% of plant-derived traditional medicines for their primary healthcare [2]. Medicinal plants and natural products extracted from them have been utilized as a source of medicines since ancient times across the world for treating and preventing different human diseases like cancer, diabetes, urinary disorders, skin diseases, etc. [3]. The current medical treatments against different human diseases have several undesired adverse side effects. Traditional medicines involve the use of different plant parts like seeds, leaves, bark, flowers, stems, etc., or their extracts or bioactive compounds for medicinal purposes. They are easily available, inexpensive, safe, and have minimal or no side effects [4]. The different bioactive constituents of medicinal plants are also called secondary metabolites, such as alkaloids, flavonoids, tannins, terpenoids, etc. These compounds possess therapeutic significance in various areas of medicine and have antioxidant, antimicrobial, and anticancer activities, among others [5, 6].

Nepal is a huge repository of medicinal plants because of its geographical and climatic diversity and its location in the central Himalayan region [7]. About 10 percent of the expected 7000 species of flowering plants in Nepal are reported to be medicinal [7]. In another study, 1792 species of plants in Nepal are reported to be of medicinal value [8]. Hence, there is a chance of finding medicinal plants having potential therapeutic significance against various diseases such as cancer, malaria, tuberculosis, and diabetes, among others.

Mahonia napaulensis DC. (synonym *Berberis nepalensis* (DC.) Spreng.) belongs to the Berberidaceae family. It is a perennial evergreen shrub with yellow flowers in winter and widely distributed in the high mountainous regions from Nepal to Bhutan, northeast India, China, and Vietnam at about 1000 m – 2000 m altitude. It is also known as Jamanemandro in Nepali, Michiki swan in Newari, and Mahonia in English [8, 9]. The stem and bark of this plant have anti-inflammatory, antibacterial, antifungal, antioxidant activity, etc. It is particularly used for the treatment of skin diseases like eczema, psoriasis, etc. It is a traditionally essential flower for conducting Bel Bibaha and Bratabandha in the Newar community [9]. The bark has antidysentric and antidiarrheal activity. The bark juice is used during the irritation of eyes [8]. The methanolic extract of *M. napaulensis* bark showed antimicrobial and antioxidant properties. The methanol solution of the bark has phytochemicals like terpenoids, reducing sugars, tannins, alkaloids, glycosides including cardiac glycosides and steroids [10]. R. Chatterjee and Govindachari et al., (1957) reported the isolation of two alkaloids: umbellatine and neprotine from *Mahonia napaulensis* root and stem bark [11, 12]. The antimicrobial activity of the methanolic extract of *M. napaulensis* bark might be due to the presence of alkaloids and flavonoids [13].

The present study was conducted for the purpose of phytochemical screening and chemical composition analysis, to determine the total phenolic and flavonoid contents, and to evaluate the antioxidant and biological (especially antibacterial, antifungal, and anticancer) activities of methanol extract of *M. napaulensis* DC. bark (MNBM). Based on our information, this is the first study evaluating the anticancer activity of methanol extract of *M. napaulensis* DC. bark and its chemical composition by LC–MS/MS.

Materials and methods

The schematic framework of the research study is shown in Fig. 1.

Sample collection and authentication

The plant bark sample was collected from Tinjure Danda, Tehrathum District, Nepal in May 2019. A plant herbarium specimen was deposited at National Herbarium and Plant Laboratories (KATH), Godawari-3, Lalitpur, Nepal and the voucher specimen number is KATH167089. The plant was identified as *Mahonia napaulensis* DC. (synonym: *Berberis nepalensis* (DC.) Spreng.). The plant was identified by Til Kumari Thapa, a Research Officer of National Herbarium and Plant Laboratories (KATH), Godawari-3, Lalitpur, Nepal (https://kath.gov.np/authorities). The permission for the plant sampling and its research analysis, both in Nepal and abroad, has been granted by Kalpana Sharma Dhakal, a Scientific Officer from the Department of Plant Resources, Thapathali, Kathmandu, under the Ministry of Forests and Environment, Government of Nepal (https://dpr.gov.np/en/about-us/staff-2/#teammodal245214).

Plant material and extraction

The plant bark was cleaned and shade dried. The dried plant bark was ground into fine powder. The extraction was done by cold maceration process using methanol solvent in a closed vessel for three days at room temperature. The extract was collected. This process was repeated three times consecutively for the same plant material. The extract was filtered using Whatman filter paper No. 1. The filtered extract was dried under reduced pressure at a temperature below 40°C using a rotary evaporator

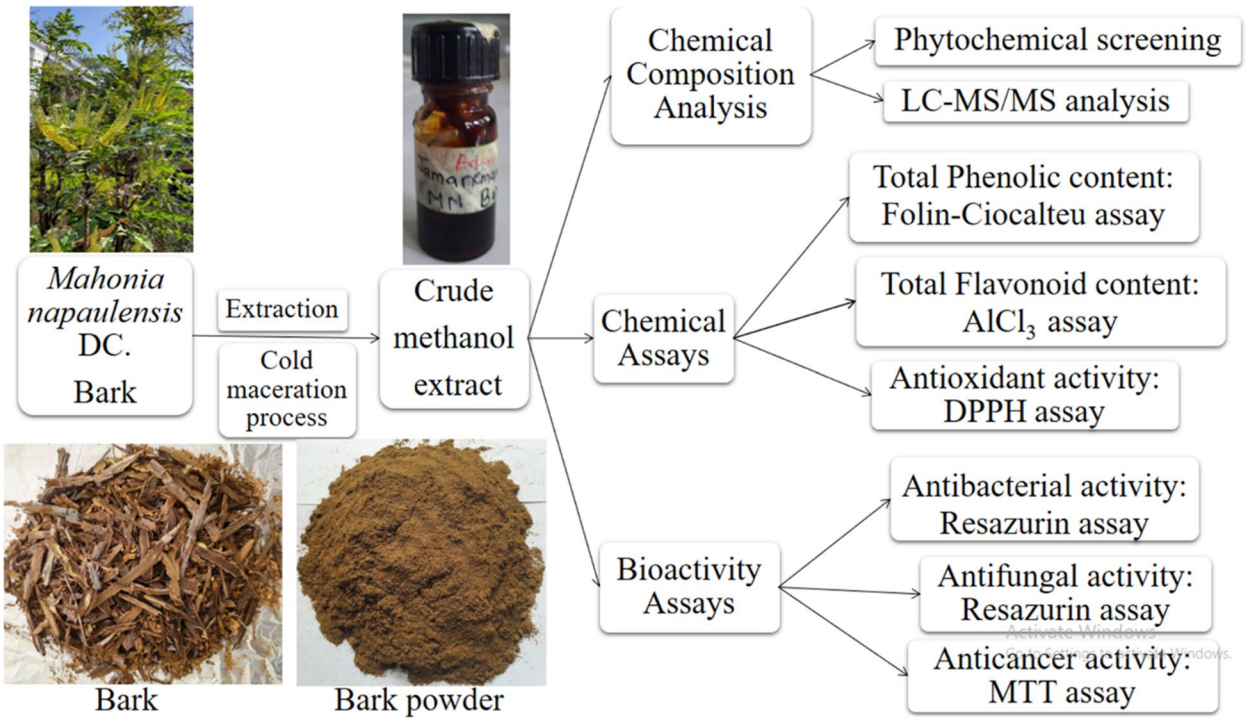

Fig. 1 Schematic framework of the research study

(BIOBASE RE-2000B Rotary Evaporator, China) and then, by using a freeze dryer or lyophilizer. Then, the extract was stored in a closed vial at 4°C for further analysis.

Phytochemical screening of methanol extract

The preliminary phytochemical screening of the plant extract was carried out by following the standard protocol of the references [14–18].

Alkaloid test: Mayer's reagent test

The crude extract was taken. 2 mL of 1% HCl was added and gently heated, followed by the addition of few drops of Mayer's reagent. The appearance of a dull white precipitate (turbidity of resulting precipitate) indicates the presence of alkaloids.

Flavonoids test: Shinoda test

A few fragments of magnesium (Mg) ribbon were added to the crude extract, followed by the addition of conc. HCl dropwise (5–6 drops). The appearance of pink or magenta red colour after a few minutes indicates the presence of flavonoids.

Terpenoids test

The crude extract was taken and 2 mL chloroform ($CHCl_3$) was added. The mixture was evaporated to dryness, followed by the addition of 2 mL conc. H_2SO_4. The presence of reddish-brown coloration at the interface indicates the presence of terpenoids.

Polyphenols and Tannins test: 2% $FeCl_3$ test

The crude extract was taken and 2 mL of 2% $FeCl_3$ was added. The appearance of blue-green/blue-black coloration indicates the presence of polyphenols and tannins.

Steroids test

The crude extract was taken. 2 mL chloroform ($CHCl_3$) was added, followed by few drops of conc. H_2SO_4 and then, 2 mL conc. acetic acid. The development of greenish colouration indicates the presence of steroids.

Glycosides test: Salkowski's test

2 mL chloroform ($CHCl_3$) was added to the crude extract and then, 2 mL conc. H_2SO_4 was added. The solution was shaken gently. The appearance of reddish brown colour indicates the presence of a steroidal ring of glycine portion of the glycoside.

Coumarins test

The crude extract was taken and hot water was added. Then, it was cooled and split into two test tubes. One test tube was taken as control. In the second test tube, 10% NH_4OH was added. The appearance of fluorescence indicates the presence of coumarins.

Quinone test

The crude extract was taken and 1 mL conc. H_2SO_4 was added. The formation of the red colour indicates the presence of quinone.

Saponins test: Froth/Foam test

The crude extract was dissolved in 5 mL distilled water and vigorously shaken for 30 s. Even after 30 min, the stable foam (1 cm height) was not formed, which indicates the absence of saponins.

Protein test: Ninhydrin test

The crude extract was taken and 2 mL of 0.25% Ninhydrin solution was added. The violet colour was not formed, which indicates the absence of protein.

Total phenolic content (TPC) determination

The total phenolic content (TPC) in plant extract was determined following Folin-Ciocalteu reagent colorimetric assay using 96-well plates [19, 20]. The experiment was performed in triplicate.

The standard Gallic acid solution of different concentrations (200, 150, 100, 50, 25, and 12.5 µg/mL) was prepared to generate a calibration curve. The plant extract sample solution of a single concentration of 500 µg/mL was prepared.

The standard or sample solution of 20 µL was taken in each well. 100 µL Folin-Ciocalteu reagent was added and mixed well. After 5 min, 80 µL sodium carbonate solution (75 g/L) was added and mixed well. The plate was left in the dark at room temperature for 2 h. It was shaken for 60 s before reading. The absorbance was measured at 750 nm in the Biotek epoch microplate reader. The standard and sample were measured against a methanol reagent blank.

The concentration of phenolic compounds in the plant extract was determined from the standard Gallic acid calibration curve. Then, the total phenolic content (TPC) was expressed as milligram Gallic acid equivalent (GAE) per gram dry sample using the formula.

$$\text{Total phenolic content (TPC)} = \frac{C \times V}{m}$$

Where,

C = Concentration of TPC in a plant extract sample solution

V = Volume of plant extract sample solution prepared

m = Amount of extract in plant extract sample solution prepared

Total flavonoid content (TFC) determination

The total flavonoid content (TFC) in plant extract was evaluated by aluminium chloride ($AlCl_3$) colorimetric method using 96-well plates [19, 21]. The experiment was performed in triplicate.

The standard Quercetin solution of different concentrations (500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL) was prepared to generate a calibration curve. The plant extract solution of a single concentration of 500 µg/mL was prepared.

To each well, 100 µL distilled water was added, followed by 10 µL of $NaNO_2$ (50 g/L) and 25 µL of standard or sample solution. After 5 min, 15 µL of $AlCl_3$ (100 g/L) was added to the mixture. Then, 6 min later, 50 µL NaOH (1 M) and 50 µL distilled water were added. The plate was shaken for 30 s and absorbance reading was taken at 510 nm in the Biotek epoch microplate reader. The standard and sample were measured against a methanol reagent blank.

The concentration of flavonoid compounds in the plant extract was determined from standard Quercetin calibration curve. Then, the total flavonoid content (TFC) was expressed as milligram Quercetin equivalent (QE) per gram dry sample using the formula.

$$\text{Total flavonoid content (TFC)} = \frac{C \times V}{m}$$

Where,

C = Concentration of TFC in a plant extract sample solution

V = Volume of plant extract sample solution prepared

m = Amount of extract in plant extract sample solution prepared

Antioxidant assay

The antioxidant activity of plant extract was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [19, 22–24]. The experiment was performed in triplicate using 96-well plates. Firstly, 100 µL of plant extract of different concentrations (250, 200, 100, 50, and 25 µg/mL) were taken in all the wells and then, 100 µL of DPPH solution (39.4 µg/mL) was added in each well. The mixture was placed in the dark for 30 min at room temperature. Then, absorbance reading was taken in 517 nm wavelength in the Biotek epoch micro-plate reader. Ascorbic acid (15, 10, 5, 2.5, and 1.25 µg/mL) was used as the positive control and methanol as the negative control. The DPPH free radical scavenging activity was calculated using the following formula.

$$\%\ \text{radical scavenging} = \left(\frac{A_o - A_t}{A_o}\right) \times 100\%$$

where, A_0 = Absorbance of Methanol with DPPH solution

A_t = Absorbance of Test samples or Positive control

Antimicrobial assay

Firstly, the antimicrobial screening of MNBM was performed by agar plate well diffusion method and then, its antimicrobial activity was measured quantitatively by Resazurin test.

Test microorganisms and Standard

Four bacterial strains and one fungal strain were taken for the antimicrobial potency of the plant extract. They were obtained from Zest Laboratories & Research Center (P.) Ltd., Balkot, Bhaktapur, Nepal. Two bacterial strains of Gram-positive: *Bacillus cereus* (ATCC: 11778) and *Staphylococcus aureus* (ATCC: 6538) and Gram-negative: *Escherichia coli* (ATCC: 8739) and *Pseudomonas aeruginosa* (ATCC: 9027) were taken. Also, one fungal strain, *Candida albicans* (ATCC: 10231) was taken. Gentamicin as a standard drug (positive control) and DMSO (0.1%) without plant extract as the negative control were used.

Antimicrobial screening: Agar plate well diffusion method

The agar plate well diffusion method was used for antimicrobial screening following the standard protocol [22, 24–27]. The experiment was done in duplicate.

The Mueller Hinton agar (MHA) was dissolved and autoclaved at 121°C for 15 min, cooled up to 45°C and then, 40–50 mL media was poured in a sterile 14 cm diameter Petri plate, and allowed to solidify and kept at room temperature.

Preparation of plant extract

The plant extract test solutions were prepared in DMSO for antimicrobial screening. A concentration of 120 mg/mL was prepared for antimicrobial screening against *S. aureus, B. cereus*, and *P. aeruginosa*. A concentration of 80 mg/mL was prepared for antimicrobial screening against *E. Coli* and *C. albicans*.

Preparation of inoculums

The bacterial and fungal strains were sub-cultured overnight at 37°C in Soyabean Casein Digest Medium (SCDM) and Sabouraud Dextrose Agar (SDA) respectively. The microbial growth was harvested and diluted to attain a viable cell count of 0.5 McFarland (1.5×10^8 CFU/mL) for bacteria and 2 McFarland (6×10^8 CFU/mL) for fungi using a spectrophotometer.

Inoculation

For inoculation, swabbing was done with the help of sterile cotton. The desirable concentration of the plant extract was added to the wells of the plate, and incubated at 37°C for 24 h. After incubation, the diameter of the zone of inhibition was measured.

Determination of Minimum Inhibitory Concentrations (MICs): Resazurin test

The Minimum Inhibitory Concentrations (MICs) of MNBM against *E. coli*, *S. aureus*, and *C. albicans* were determined by Resazurin test.

Preparation of plant extract for Resazurin test

The plant extract concentration of 200.43 mg/mL was prepared in DMSO for MIC test against *S. aureus* and 100.3 mg/mL was prepared in DMSO for MIC test against *E. coli* and *C. albicans*.

Preparation of resazurin solution

A resazurin solution of 6.75 mg/mL was prepared in sterile water, vortexed, and filtered by 0.22 µm filter. Then, it was stored at 4°C for further use.

Preparation of standard Gentamicin

The standard solution of Gentamicin of 0.076 mg/mL concentration was prepared for the positive control.

Preparation of standardized inoculum

The bacterial and fungal strains were sub-cultured overnight at 37°C in Soyabean Casein Digest Medium (SCDM) and Sabouraud Dextrose Agar (SDA) respectively. The microbial growth was harvested and diluted to attain a viable cell count of 0.5 McFarland (1.5×10^8 CFU/mL) for bacteria and 2 McFarland (6×10^8 CFU/mL) for fungi using a spectrophotometer.

Resazurin test using 96-well plates

MIC was determined by following the standard protocol [28, 29] with some modifications. The experiment was done in triplicate.

All the wells of a sterile 96-well plate were labeled as required. Firstly, 100 µL SCDM was kept in all the wells. Then, 100 µL of plant extract was kept in the first row wells in triplicate and then, serially diluted. Similarly, 100 µL of gentamicin in the first well of the positive control line and 100 µL of DMSO in the first well of the negative control line were kept and serially diluted. After that, 100 µL test microorganism was added in all the wells of plant extract and also, in the positive and negative control line. At last, 10 µL resazurin indicator was added in all the wells. Then, it was covered with aluminium foil and incubated at 37°C for 24 h and the result was observed visually. The colour change from blue/purple to pink or colourless was recorded as positive. The lowest

concentration prior to colour change was taken as the Minimum Inhibitory Concentration (MIC) value.

Anticancer activity

The anticancer activity of plant extract against different human cancer cells was evaluated by MTT colorimetric assay in 96-well plates following the standard protocol of references [18, 22, 23, 30, 31].

Cell culture

Different human cancer cell lines were provided by the Cancer Biology Laboratory, School of Life Sciences (SLS), Jawaharlal Nehru University (JNU), New Delhi, India, and Department of Biotechnology, Kathmandu University, Nepal. The Cancer Biology Department, JNU provided A549 human lung cancer cells (ATCC: CCL-185, Manassas VA). The Department of Biotechnology, Kathmandu University, Nepal provided HeLa human cervical cancer cells (ECACC-93021013) which were acquired from Shikhar Biotech Pvt. Ltd., Khumaltar Height, Ward No. 15, Lalitpur, Nepal.

The human cancer cells, A549 and HeLa, were cultured in DMEM medium (Dulbecco's Modified Eagle Medium) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (HIMEDIA, India) and 1% antibiotics (penicillin & streptomycin) (HIMEDIA, India) at 37°C in a humidified 5% CO_2 incubator until 80% confluency. The confluence was observed under an inverted microscope and sub-cultured at three to four days intervals.

In vitro cell viability assay: MTT assay

After 80% confluence, the media was discarded and the cells were washed with phosphate buffer saline (PBS). The cells were trypsinized and suspended in fresh media. Then, cell counting was done using a hemocytometer. 1×10^4 cells were seeded in 96-well plates and incubated for 24 h. After 24 h, media from the wells was removed and treated with different concentrations (25–400 µg/mL) of MNBM prepared in the media. The negative control was DMSO (0.1%) and the positive control was fisetin (75 µM) (Sigma-Aldrich). Then, the cells were incubated for 48 h. After 48 h, media were discarded and 100 µL MTT (0.5 mg/mL) in media was added and incubated for 4 h. After 4 h incubation, MTT dye will be converted to formazan which is water insoluble. Then, the media was removed and replaced with 100 µL DMSO in each well to dissolve formazan. The absorbance reading was taken in a microplate reader (Thermo Scientific MULTISKAN GO) at 570 nm wavelength. Each test was done in triplicate.

The cytotoxicity results were recorded as IC_{50} value (concentration causing 50% growth inhibition for the cell lines). The cell viability was calculated by the following formula,

$$\%\ \text{Cell viability} = \left(\frac{A_t}{A_o}\right) \times 100\%$$

Where, A_o = Absorbance of the cell line without plant extract treatment

A_t = Absorbance of the cell line with plant extract treatment

Metabolite profiling of MNBM by LC–MS/MS

The liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis was performed in VProteomics Laboratory, Valerian Chem Pvt. Ltd., Gautam Buddha Nagar, Noida, Uttar Pradesh—201,301, India.

Experimental procedure

Sample preparation

The sample was dissolved in 200 µL of 80% MeOH and mixed with a Vortexer. The samples were mixed for 5 min and centrifuged at 13,000 × g for 5 min to remove solid particles from the supernatant. 100 µL supernatant was transferred into a 150 µL glass insert in a 1.5 mL amber glass vial and analysed by UHPLC-MS/MS.

Liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS)

All LC-MS/MS experiments were carried out using the Dionex UltiMate 3000 UHPLC (Thermo Scientific, MA) system equipped with a binary solvent delivery manager and a sample manager, coupled with a Thermo Q-EXACTIVE PLUS operating in positive (ESI+) and negative (ESI−) electrospray ionization mode (Thermo Fisher Scientific, Sunnyvale, CA, USA), separately. The liquid chromatography was a Thermo Hypersil Gold C18 column (100 mm × 2.1 mm, 3 µm particle size). Separation was achieved with solvent A (Water + 0.1% (v/v) formic acid) and solvent B (acetonitrile + 0.1% (v/v) formic acid) with the following gradient at a flow rate of 0.3 mL/min: 0 min (5% B), 0–8 min (5–95% B), 8–11 min (95% B), 11–12 min (95–5% B) and 12–15 min (5% B). The injection volume was 5 µL and the analysis time was 15 min. The mass spectrometric data were collected using a Thermo Q-EXACTIVE PLUS mass spectrometer.

Data processing

All samples were processed and RAW files generated were analysed with Compound Discoverer (CD)—3.3. For compound consolidation, Mass Tolerance was set at 10 ppm. Metabolite annotation was performed through ChemSpider and mzCloud databases. ChemSpider database search was performed with BioCyc, Food and Agriculture Organization of the United Nations, KEGG,

Plant Cyc, Planta Piloto de Quimica Fina, Universidad de Alcala, PlantCyc.

The results of the analysis were exported with the following filtration criteria: delta ppm range from −10 to 10 ppm, name is fully annotated, and for which all annotations have MS/MS. Both positive and negative mode analysis were performed separately, which was combined at a later stage, and duplicates were removed.

Statistical analysis

The experiments were done in triplicate. The results were expressed as mean ± standard deviation of mean determined by Microsoft Excel and Origin 7.5. The statistical significance of differences between test groups was analyzed by one-way analysis of variance (ANOVA: Single Factor). The differences were considered statistically significant where P values < 0.05.

Results

Extraction yield percentage

The extraction yield of methanol extract from dried plant bark powder sample obtained by the cold maceration process was calculated in percentage. The extraction yield percentage was calculated by using the following formula.

$$\text{Extraction Yield \%} = \frac{\text{Weight of the plant extract obtained}}{\text{Total weight of the sample used for extraction}} \times 100 = \frac{8.58}{100} \times 100 = 8.58\%$$

The yield of the methanol extract (orange coloured) obtained from 100 g dried plant bark was 8.58 g (8.58% w/w) as shown in Table 1.

Total Phenolic content (TPC)

The total phenolic content in MNBM was determined by Folin-Ciocalteu reagent colorimetric assay using Gallic acid as standard and expressed as milligram Gallic acid equivalent (GAE) per gram dry MNBM sample. The result was calculated from the standard Gallic acid calibration curve ($R^2 = 0.99887$) using Origin 7.5 (Fig. 2).

Table 1 The extraction yield %, TPC, TFC, and antioxidant activity of MNBM

Analysis/activity	MNBM
Extraction Yield %	8.58% (w/w)
TPC	38.00 ± 1.50 mg GAE g^{-1} dry sample
TFC	35.04 ± 4.87 mg QE g^{-1} dry sample
Antioxidant activity (IC_{50} value)	212.97 µg/mL

The plant extract was found to contain the TPC of 38.00 ± 1.50 mg GAE per gram of dry plant bark extract.

Total Flavonoid content (TFC)

The total flavonoid content in MNBM was measured by the aluminum chloride colorimetric assay using Quercetin as standard and expressed as milligram Quercetin equivalent (QE) per gram dry MNBM sample. The result was determined using a linear calibration curve of Quercetin ($R^2 = 0.99492$) using Origin 7.5 (Fig. 3). The plant extract was found to contain the TFC of 35.04 ± 4.87 mg QE per gram of dry plant extract.

Antioxidant activity

The percentage scavenging activity and IC_{50} values of MNBM as sample and ascorbic acid as standard were assessed by plotting the regression line curve using Origin 7.5.

The MNBM showed dose-dependent scavenging activity 11.39 ± 1.06% at 25 µg/mL to 57.00 ± 1.58% at 250 µg/mL, with 212.97 µg/mL IC_{50} value ($P < 0.05$) as shown in Table 1. The standard ascorbic acid showed dose-dependent scavenging activity 5.54 ± 0.57% at 1.25 µg/mL to 87.12 ± 0.57% at 15 µg/mL, with 8.28 µg/mL IC_{50} value ($P < 0.05$). The comparison of percentage scavenging activity of MNBM with standard ascorbic acid is shown in Fig. 4. The comparison of antioxidant activity (IC_{50} value) of MNBM with standard ascorbic acid is shown in Fig. 5. The methanol extract of *M. napaulensis* bark (MNBM) showed moderate antioxidant activity. The antioxidant activity of the plant extract is considered as very strong ($IC_{50} < 50$ µg/mL), strong (IC_{50}: 50–100 µg/mL), moderate (IC_{50}: 101–250 µg/mL), weak (IC_{50}: 250–500 µg/mL) and inactive (IC_{50}: > 500 µg/mL) [32].

Antimicrobial activity

Antimicrobial screening: Agar plate well diffusion method

The antimicrobial screening was assessed by measuring the zone of inhibition (ZOI) of different concentrations on the agar plate and the results are shown in Table 2. The plant bark methanol extract (MNBM) showed no antimicrobial effect against *B. cereus* and *P. aeruginosa* at 120 mg/mL. However, the plant showed antimicrobial activity towards bacteria with ZOI of 27 mm for *E. coli* at 80 mg/mL concentration and ZOI of 27 mm for *S. aureus* at 120 mg/mL concentration. Its antimicrobial activity towards fungus with ZOI of 23.5 mm for *C. albicans* at 80 mg/mL concentration. Thus, the methanol extract of *M. napaulensis* bark is very active against *E. coli*, *S. aureus*, and *C. albicans*, but resistant to *B. cereus* and *P. aeruginosa*. The results of the

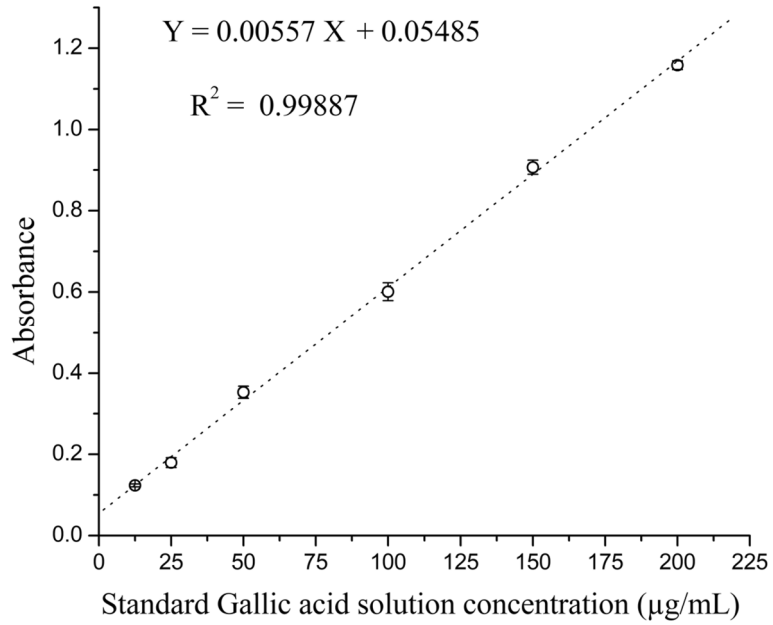

Fig. 2 Determination of total phenolic content (TPC) in MNBM using standard Gallic acid calibration curve by Folin-Ciocalteu colorimetric assay (*Significant differences are indicated by $P < 0.05$ as compared with control.)

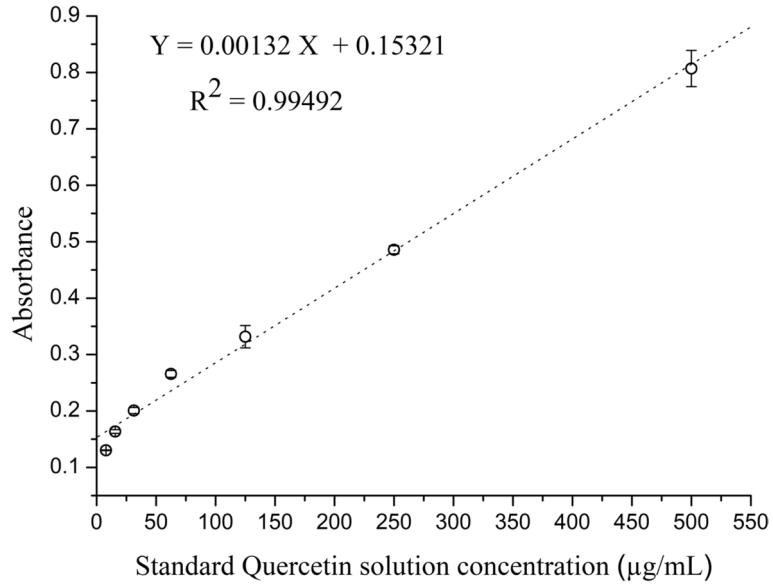

Fig. 3 Determination of total flavonoid content (TFC) in MNBM using standard Quercetin calibration curve by $AlCl_3$ colorimetric assay (*Significant differences are indicated by $P < 0.05$ as compared with control.)

antimicrobial activity in terms of ZOI were expressed as the sample is inactive (ZOI < 9 mm), partially active (ZOI = 9–12 mm), active (ZOI = 13–18 mm), and very active (ZOI > 18 mm) [27, 33].

The antimicrobial screening result showed that both *B. cereus* and *P. aeruginosa* were the most resistant strains to the plant bark methanol extract. *E. coli, S. aureus,* and *C. albicans* were the most susceptible strains to the plant bark methanol extract. Hence, an experiment was conducted to determine the minimal inhibitory concentration (MIC) against the most susceptible bacterial strains (*S. aureus* and *E. coli*) and fungal strain (*C. albicans*).

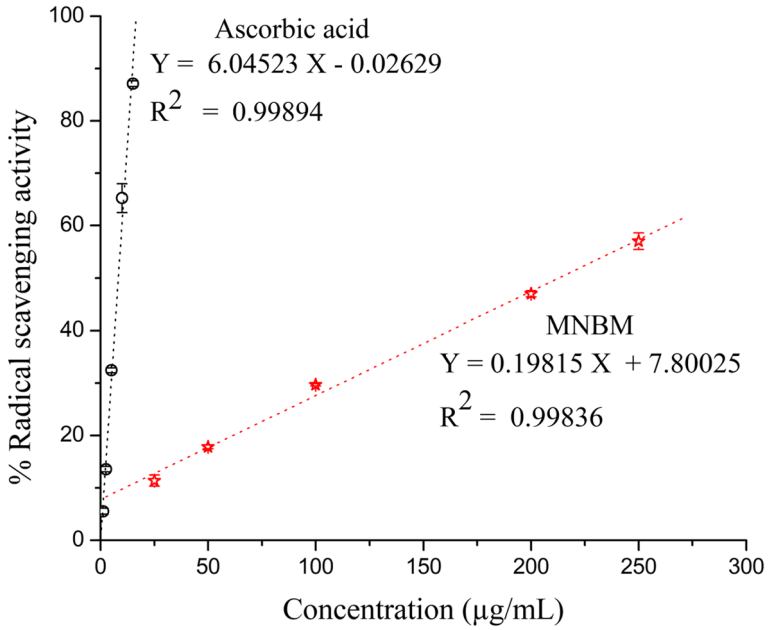

Fig. 4 Comparison of percentage radical scavenging activity between ascorbic acid and MNBM (*Significant differences are indicated by $P < 0.05$ as compared with control.)

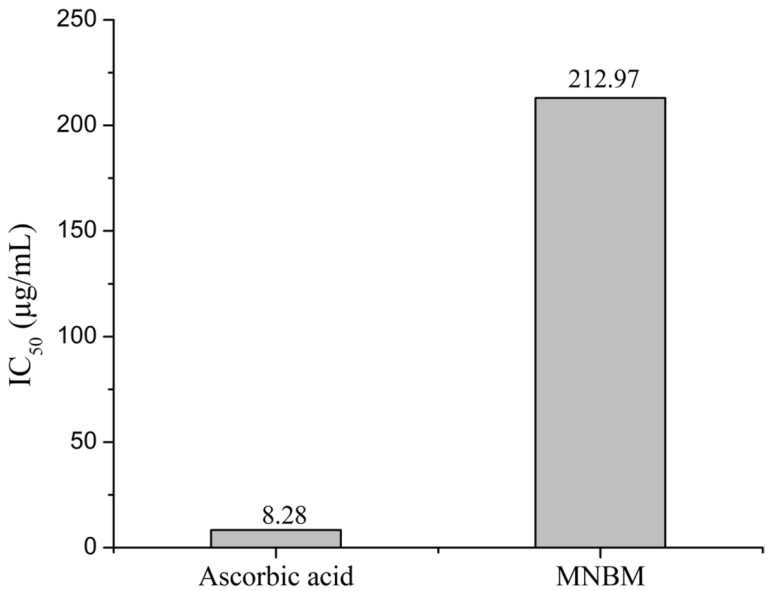

Fig. 5 The comparison of antioxidant activity (IC_{50} value) of ascorbic acid and MNBM

Determination of Minimum Inhibitory Concentrations (MICs): Resazurin test

The lowest concentration of MNBM prior to the colour change from blue/purple to pink was taken as the MIC value and the results are shown in Table 2.

The MIC values of MNBM against bacterial strains were determined as 100.22 mg/mL for *S. aureus* and 50.15 mg/mL for *E. coli*. The MIC value against fungal strain *C. albicans* was 25.08 mg/mL. The MIC value for standard Gentamicin solution was recorded as 0.42 µg/mL against *S. aureus*. The methanol extract of *M. napaulensis* bark (MNBM) showed weak antimicrobial activity. The antimicrobial activity of the plant extracts is considered as significant (MIC < 100 µg/mL), moderate (100 < MIC ≤ 625 µg/mL), and weak (MIC > 625 µg/mL) [34, 35].

Table 2 Zone of Inhibition (ZOI) and MIC values of MNBM

Plant Extract	Microbes	MIC (mg/mL): Resazurin test	Anti-microbial screening: Agar plate well diffusion method			
			Extract concentration (mg/mL)	Zone of Inhibition (ZOI) (mm)		
				S_1	S_2	Average
MNBM	*E. coli* (bacteria)	50.15	80	26	28	27
	P. aeruginosa (bacteria)	-	120	resistant	resistant	resistant
	S. aureus (bacteria)	100.22	120	26	28	27
	B. cereus (bacteria)	-	120	resistant	resistant	resistant
	C. albicans (fungus)	25.08	80	23	24	23.5
Standard Gentamicin solution		0.42 µg/mL				

Table 3 *In vitro* anticancer activity (IC_{50} value) of MNBM

Plant Extract	Human cancer cell lines	IC_{50} (µg/mL)
MNBM	A549	228.93
	HeLa	367.72

Anticancer activity

M. napaulensis bark methanol extract (MNBM) showed dose-dependent anticancer activity against different human cancer cell lines analyzed by *in vitro* MTT assay, and *in vitro* IC_{50} values are shown in Table 3.

MNBM showed the cell viability 81.65 ± 4.26% at 25 µg/mL and 33.87 ± 0.47% at 300 µg/mL against A549 lung cancer cells as shown in Fig. 6, with 228.93 µg/mL IC_{50} value ($P < 0.05$). MNBM showed the cell viability 84.14 ± 3.87% at 50 µg/mL and 45.11 ± 4.44% at 400 µg/mL against HeLa cervical cancer cell line as shown in Fig. 7, with 367.72 µg/mL IC_{50} value ($P < 0.05$). Figure 8 shows the comparison of cell viability for MNBM against A549 and HeLa cancer cells. MNBM showed less IC_{50} value and thus more anticancer activity against A549 cells in comparison to HeLa cells. Overall, MNBM showed moderate anticancer activity against A549 and HeLa cells. The anticancer activity of the plant extract is considered potential ($IC_{50} < 100$ µg/mL), moderate (100 µg/mL < $IC_{50} < 1000$ µg/mL), and nontoxic ($IC_{50} > 1000$ µg/mL) [36].

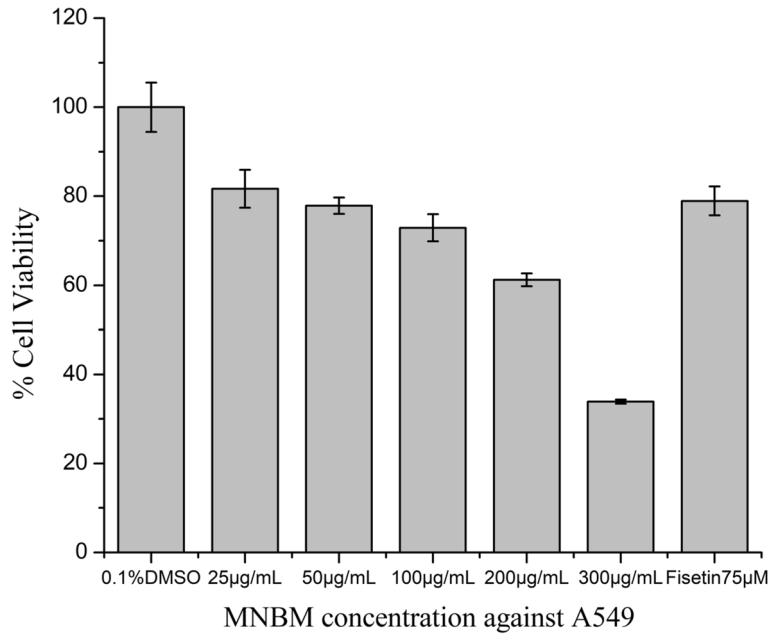

Fig. 6 Comparison of percent cell viability shown by MNBM against A549 cells (ATCC: CCL-185) with 0.1% DMSO (as negative control) and Fisetin 75 µM (as positive control) analyzed by *in vitro* MTT assay (*Significant differences are indicated by $P < 0.05$ as compared with controls.)

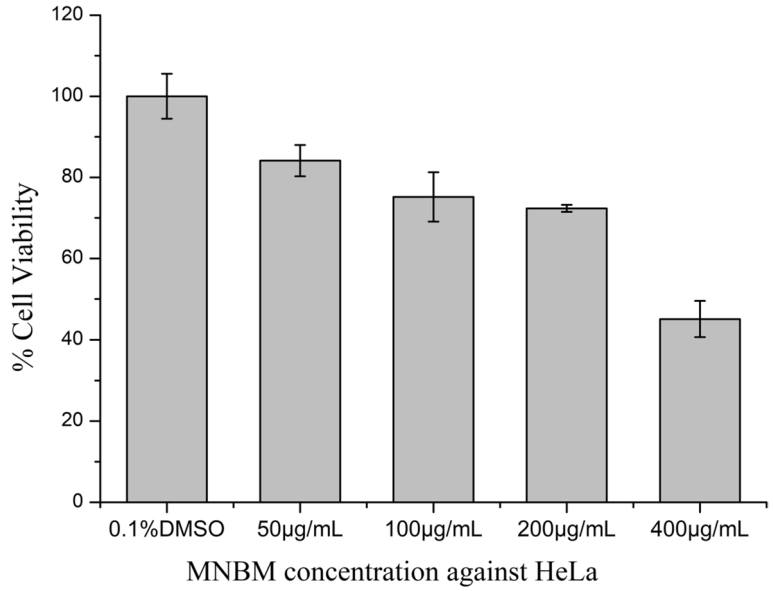

Fig. 7 Comparison of percent cell viability shown by MNBM against HeLa cells (ECACC-93021013) with 0.1% DMSO (as negative control) analyzed by *in vitro* MTT assay (*Significant differences are indicated by $P < 0.05$ as compared with control.)

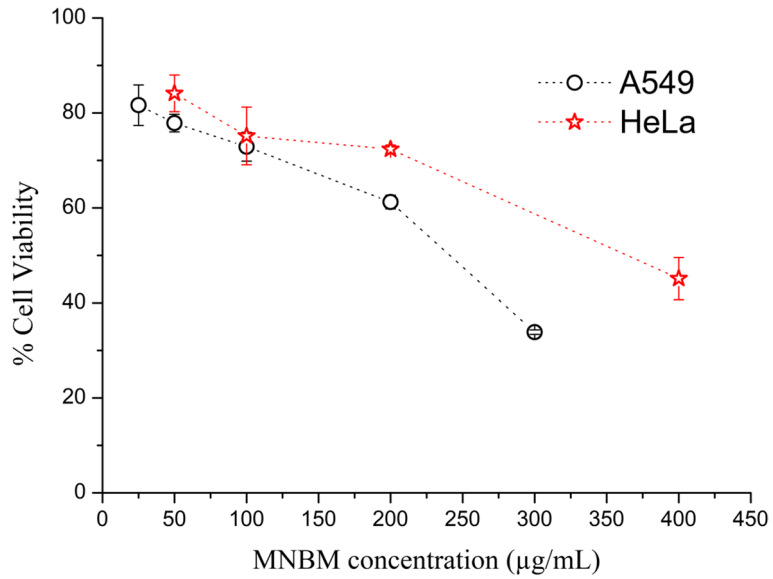

Fig. 8 Comparison of percent cell viability shown by MNBM against A549 and HeLa cancer cell lines (*Significant differences are indicated by $P < 0.05$ as compared with controls.)

Chemical composition of MNBM

Phytochemical screening of methanol extract

The qualitative phytochemical screening of the methanol extract of plant bark (MNBM) revealed the presence of various phytochemicals like alkaloids, steroids, glycosides, polyphenols, tannins, flavonoids, coumarins, terpenoids, and quinone, but the absence of saponins and protein. The results are shown in Table 4.

Table 4 Results of phytochemical screening of MNBM

Phytochemicals	MNBM
Alkaloids	+
Steroids	+
Glycosides	+
Polyphenols & tannins	+
Flavonoids	+
Coumarins	+
Terpenoids	+
Quinone	+
Saponins	-
Protein	-

Metabolite profiling of MNBM by LC–MS/MS

The metabolite profiling of methanol extract of *M. napaulensis* bark (MNBM) was done by LC–MS/MS analysis and it identified 507 compounds. The chemical contents, retention time, and area percentage of the most abundant 59 metabolites are sorted in Table 5 according to maximum to minimum abundance order. The most abundant five metabolites are dihydroberberine (34.25%), ethylmorphine (8.91%), 2-(methylthiomethyl)−3-phenylacrylaldehyde (8.57%), ochotensine (7.29%) and d-berbamine (6.56%). The literature study through PubChem and Chemical Entities of Biological Interest (ChEBI) reported that the metabolites dihydroberberine (34.25%), d-berbamine (6.56%), (S)-glaucine (1.02%), protopine (0.54%), grosheimin (0.36%), mycophenolic acid (0.18%), 5-O-methylembelin (0.14%), berberine (0.12%), (-)-(S)-equol (0.11%), alpha-linolenic acid (0.09%), polidocanol (0.08%), juglone (0.07%), lapachol (0.05%), solasodine (0.05%), podophyllotoxin (0.05%), tandutinib (0.04%), mitoxantrone (0.03%), methyl 3-indolylacetate (0.03%), eugenol (0.02%), apaziquone (0.02%), and hymecromone (0.01%) have anticancer activity.

Discussion

The previous study has reported higher stem and leaf methanol extract yields as compared to bark extract (MNBM) yield as observed in this study [9]. It reported 0.53% hexane, 4.2% ethyl acetate, and 12.6% methanol extract yields of plant stem and 4.6% hexane, 6.8% ethyl acetate, and 22.4% methanol extract yields of plant leaf obtained by the successive extraction of the plant stem and leaf on increasing polarity using the soxhlet apparatus [9].

Earlier studies have reported almost similar phytochemicals in bark methanol extract as in this study [10, 13]. The phytochemical screening revealed the presence of alkaloids, tannins, terpenoids, cardiac glycoside, saponins, steroids, and reducing sugar as phytochemicals of the methanol extract of the plant bark extracted by using the soxhlet apparatus [10]. Similarly, the presence of phytochemicals viz. alkaloids and flavonoids are reported in the methanol extract of the plant bark obtained by cold maceration process [13]. The phytochemical screening of plant stem and leaf methanol extract revealed the presence of more numerous phytochemicals viz. quinone, glycoside, flavonoid, terpenoids, alkaloid, saponins, cardiac glycoside, and steroid than in hexane and ethyl acetate extract [9]. Further, past studies have reported the IC_{50} value for antioxidant activity of methanol extract of the plant bark to be 230.89 µg/mL as determined by DPPH assay [10]. This antioxidant IC_{50} value is slightly more than that observed in this study.

The previous studies on the plant bark methanol extract showed relatively similar antimicrobial activity as in this study [9, 10, 13]. The antibacterial study on the plant bark methanol extract was reported to be 27.3 mm ZOI against *S. aureus*, 23 mm ZOI against *E. coli*, 25 mm ZOI against *P. aeruginosa*, 20 mm ZOI against *S. typhi*, and 23.6 mm ZOI against *Shigella spp.* at 10 mg/mL methanol extract concentration [10]. Similarly, another study reported 12.67 ± 1.52 mm ZOI against *S. aureus*, 7.67 ± 0.57 mm ZOI against *E. coli*, and 5 ± 1 mm ZOI against *S. typhi* at 9% bark methanol extract concentration [13]. The antibacterial study on the stem and leaf of the plant showed that hexane and ethyl acetate extract of stem and all extracts (hexane, ethyl acetate, and methanol) of leaf were resistant to all the bacteria species tested while stem methanol extract was active only against *S. aureus* with 18 mm ZOI at 30 µL extract volume [9]. *M. napaulensis* DC. leaves methanol extract showed antifungal activity against four fungal species such as *Colletotrichum capsici* (MTCC No. 2071), *Leptosphaerulina trifolii* (MTCC No. 2328), *Alternaria brassicicola* (MTCC No. 2102), and *Helminthosporium solani* (MTCC No. 2075) at 20–100 mg/L concentration [37].

The difference in yield percentage, antioxidant, and biological activities of the same plant is due to the variation in the phytochemical composition of plant extracts which depends on different factors like altitude, seasons, age of plants, different plant parts, extraction methods, polarity of solvents used, etc. [38–41]. The profile and quantity of phytochemicals in the plant extract depend on the season, extraction method, storage conditions [38], and type of solvents used [41]. Also, the phytochemical composition of plant extract depends on the variation in altitude of plant sampling sites [39], plant species type, developmental stage, and environmental conditions during growth [40].

Medicinal plants contain secondary metabolites such as terpenes, phenolic compounds, and alkaloids. They

Table 5 The chemical contents, retention time, and area percentage of metabolites present in MNBM analyzed by LC–MS/MS

S.N	Name	Formula	Calc. MW	RT (min)	Area %
1	Dihydroberberine	$C_{20}H_{19}NO_4$	337.13	4.05	34.25
2	Ethylmorphine	$C_{19}H_{23}NO_3$	313.17	2.87	8.91
3	2-(Methylthiomethyl)-3-phenylacrylaldehyde	$C_{11}H_{12}OS$	192.06	0.98	8.57
4	Ochotensine	$C_{21}H_{21}NO_4$	351.15	5.51	7.29
5	d-Berbamine	$C_{37}H_{40}N_2O_6$	608.29	3.81	6.56
6	Methyl (2E,6E)−3,7-diethyl-9-[(2R,3S)−3-ethyl-3-methyl-2-oxiranyl]−2,6-nonadienoate	$C_{19}H_{32}O_3$	308.23	12.58	1.78
7	Benactyzine	$C_{20}H_{25}NO_3$	327.18	3.25	1.75
8	Rutacridone epoxide	$C_{19}H_{17}NO_4$	323.12	3.84	1.46
9	S-(+)-Glaucine	$C_{21}H_{25}NO_4$	355.18	3.84	1.02
10	1-Linoleoyl-2-Hydroxy-sn-glycero-3-PC	$C_{26}H_{50}NO_7P$	519.33	11.73	0.98
11	Evoprenine	$C_{20}H_{21}NO_4$	339.15	4.75	0.85
12	Promecarb	$C_{12}H_{17}NO_2$	207.13	1.01	0.77
13	Atheroline	$C_{19}H_{15}NO_5$	337.09	8.25	0.73
14	Indole-3-butyric acid	$C_{12}H_{13}NO_2$	203.09	2.75	0.72
15	Mukaadial	$C_{15}H_{22}O_4$	266.15	13.03	0.71
16	Sinomenine	$C_{19}H_{23}NO_4$	329.16	2.10	0.66
17	Codeinone	$C_{18}H_{19}NO_3$	297.14	3.16	0.65
18	3-(4-Hydroxyphenyl)propanal	$C_9H_{10}O_2$	150.07	1.03	0.59
19	MFCD00674434	$C_{20}H_{37}NO_2$	323.28	12.21	0.58
20	Fenobucarb	$C_{12}H_{17}NO_2$	207.13	1.37	0.57
21	Protopine	$C_{20}H_{19}NO_5$	353.13	3.82	0.54
22	(-)-Codeine	$C_{18}H_{21}NO_3$	299.15	3.22	0.50
23	MFCD00036904	$C_{24}H_{50}NO_7P$	495.33	12.83	0.45
24	3,4-Dihydroxy-5-{[(2E)−3-(4-hydroxyphenyl)−2-propenoyl]oxy}−1-cyclohexene-1-carboxylate	$C_{16}H_{15}O_7$	319.08	4.99	0.39
25	Thebaine	$C_{19}H_{21}NO_3$	311.15	3.23	0.38
26	(-)-Carnegine	$C_{13}H_{19}NO_2$	221.14	1.83	0.38
27	MFCD00041500	$C_{16}H_{22}O_4$	278.15	11.70	0.37
28	Phensuximide	$C_{11}H_{11}NO_2$	189.08	2.20	0.37
29	Grosheimin	$C_{15}H_{18}O_4$	262.12	7.98	0.36
30	(+)-Bicuculline	$C_{20}H_{17}NO_6$	367.11	7.29	0.35
31	Fetidine	$C_{40}H_{46}N_2O_8$	682.32	3.37	0.32
32	Stearidonic acid	$C_{18}H_{28}O_2$	276.21	12.58	0.32
33	N-Acetyl-L-glutamic acid	$C_7H_{11}NO_5$	189.06	1.27	0.32
34	2′-Norberbamunine	$C_{35}H_{38}N_2O_6$	582.27	3.67	0.31
35	9-Oxo-ODE	$C_{18}H_{30}O_3$	294.22	11.45	0.29
36	N-acetyl-L-2-aminoadipic acid	$C_8H_{13}NO_5$	203.08	2.40	0.28
37	Bellendine	$C_{12}H_{15}NO_2$	205.11	2.16	0.27
38	Androstanolone	$C_{19}H_{30}O_2$	290.22	11.89	0.27
39	CI-976	$C_{23}H_{39}NO_4$	393.29	13.15	0.23
40	2IAP3WIO1P	$C_{20}H_{23}NO_5$	357.16	3.14	0.23
41	Anacardic acid	$C_{22}H_{36}O_3$	348.27	12.87	0.20
42	D-(-)-Morphine	$C_{17}H_{19}NO_3$	285.14	3.20	0.20
43	Palmitylethanolamide	$C_{18}H_{37}NO_2$	299.28	12.67	0.19
44	Mycophenolic acid	$C_{17}H_{20}O_6$	320.13	8.34	0.18

Table 5 (continued)

S.N	Name	Formula	Calc. MW	RT (min)	Area %
45	5-O-Methylembelin	$C_{18}H_{28}O_4$	308.20	8.69	0.14
46	Berberine	$C_{20}H_{18}NO_4$	336.12	6.13	0.12
47	(-)-(S)-Equol	$C_{15}H_{14}O_3$	242.09	8.74	0.11
48	a-Linolenic Acid	$C_{18}H_{30}O_2$	278.22	11.26	0.09
49	Polidocanol	$C_{30}H_{62}O_{10}$	582.43	12.29	0.08
50	Juglone	$C_{10}H_6O_3$	174.03	6.84	0.07
51	Lapachol	$C_{15}H_{14}O_3$	242.09	10.36	0.05
52	Solasodine	$C_{27}H_{43}NO_2$	413.33	7.9	0.05
53	(-)-Podophyllotoxin	$C_{22}H_{22}O_8$	414.13	8.20	0.05
54	Tandutinib	$C_{31}H_{42}N_6O_4$	562.33	11.65	0.04
55	Mitoxantrone	$C_{22}H_{28}N_4O_6$	444.20	9.92	0.03
56	Methyl 3-indolylacetate	$C_{11}H_{11}NO_2$	189.08	1.06	0.03
57	APAZIQUONE	$C_{15}H_{16}N_2O_4$	288.11	2.19	0.02
58	Eugenol	$C_{10}H_{12}O_2$	164.08	10.57	0.02
59	Hymecromone	$C_{10}H_8O_3$	176.05	1.39	0.01

have different pharmacological properties like analgesic, antimicrobial, anti-inflammatory, anticancer, antioxidant activities, and therapeutic applications in the treatment and prevention of many ailments like diabetes, cancer, gastric ulcers, etc. [42, 43]. The plant polyphenols have antioxidant, anti-inflammatory, antimicrobial, anticancer activity, etc. [5, 44]. The plant alkaloids have antibacterial, antiviral, antifungal, anticancer, antioxidant activities, etc. [42]. The plant terpenoids showed different pharmacological properties like analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic activities, etc. [5]. The phenolic compounds and flavonoids are the major metabolites responsible for the antioxidant activity [45]. Alkaloids and polyphenols are the plant metabolites accountable for antimicrobial activity [46]. The major phytochemicals having anticancer activity have been simply categorized into alkaloids, polyphenols, saponins, tannins, and terpenoids [47]. Medicinal plants rich in antioxidants can play a role in the prevention, protection, and treatment of various chronic diseases such as asthma, cardiovascular disease, cancer, etc. [48, 49].

The previous studies reported the anticancer and other biological activities of different anticancer compounds present in MNBM. Dihydroberberine, an isoquinoline alkaloid, is a more biologically available hydrogenated derivative of berberine. Dihydroberberine has higher lipophilicity and superior beneficial biological properties than berberine. Dihydroberberine has anti-atherosclerotic, anti-inflammatory, antitumor activities, anti-adiposity, anti-hyperlipidemia, antidiabetes, etc. [50–53]. The d-berbamine, a bis-benzylisoquinoline alkaloid, has biological activities like antihypertension, antiarrhythmic, anti-inflammatory, antimicrobial, antineoplastic, antioxidant, etc. [54–56]. S-(+)-Glaucine, an aporphine alkaloid, has antitussive, antibacterial, anticancer, bronchodilatory, anti-inflammatory, antinociceptive, antihypertensive, anticonvulsant, neuroleptic-like properties, etc. [57–59]. Protopine, an isoquinoline alkaloid, has different biological activities such as antibacterial, antiviral, antifungal, antiparasitic, anticancer, anti-inflammatory activities, etc. [60]. Grosheimin, a sesquiterpene lactone, has cytotoxicity and antitumor activity [61]. Mycophenolic acid possesses antibacterial, antifungal, antiviral, anticancer, immunosuppressive, antipsoriatic properties, etc. [62, 63]. Berberine, an isoquinoline alkaloid, shows many bioactivities such as antioxidant, anti-inflammatory, antidiabetic, anti-obesity, antimicrobial, anticancer activities, etc. [64–66]. (-)-(S)-Equol (4′,7-isoflavandiol) has various properties such as anticancer, antioxidant, anti-aging, cardioprotective, antidiabetic, antiosteoporosis, neuroprotective activities, etc. [67, 68]. Alpha-linolenic acid, a polyunsaturated omega-3 fatty acid, has different pharmacological activities such as neuroprotective, cardioprotective, anti-inflammatory, anticancer, hepatoprotective, antioxidant, antidiabetic, anti-obesity, anti-osteoporotic, etc. [69–71]. Juglone (5-hydroxy-1,4-naphthoquinone) has anticancer, cytotoxic, antioxidant, antiviral, antibacterial, antifungal, antiparasitic activities, etc. [72–75]. Lapachol, a plant-derived naphthoquinone compound, has various biological properties such as antioxidant, anti-inflammatory, antibacterial, trypanomicide, leishmanicide, anticancer activity, etc. [76–78]. Solasodine performs different functions including antioxidant, anti-infection, neurogenesis promotion effects, etc. In addition, solasodine exhibits anticancer activity against human colorectal, lung, ovarian cancer

cells, etc. [79]. Podophyllotoxin, a natural lignan, has antitumor properties [80]. Tandutinib, a piperazinyl quinazoline class compound, has anticancer and antiangiogenesis activity [81]. Mitoxantrone is an anthraquinone antineoplastic agent and shows significant anticancer activity in the treatment of metastatic breast cancer, acute leukemia, lymphoma, lung cancer, prostate cancer, etc. [82, 83]. Methyl 3-indolylacetate is the methyl ester of indole-3-acetic acid and has anticancer activity [84]. Eugenol, a phenylpropanoid, has antibacterial, antiviral, antifungal, anticancer, anti-inflammatory, antioxidant properties, etc. [85]. Hymecromone or 4-methylumbelliferone, a hydroxycoumarin, has antioxidant and anticancer activity [86–88]. PubChem reported that apaziquone, a member of indoles, is used for the treatment of bladder cancer and bladder neoplasms and polidocanol is a hydroxypolyether with potential antineoplastic activities [89, 90].

Conclusion

The present study concluded that the methanol extract of *M. napaulensis* DC. bark (MNBM) showed dose-dependent moderate antioxidant, weak antibacterial, weak antifungal, and moderate anticancer activity. It showed no antibacterial effect against *B. cereus* and *P. aeruginosa*. It showed weak antifungal activity against *C. albicans* and weak antibacterial activity against *S. aureus* and *E. coli.* Moreover, it showed moderate anticancer activity against A549 and HeLa cancer cells. Its biochemical and biological activities are due to the synergistic effect of different phytochemicals such as alkaloids, total phenolic contents, total flavonoids contents, terpenoids, etc. and different anticancer compounds such as dihydroberberine, d-berbamine, (S)-glaucine, protopine, grosheimin, mycophenolic acid, 5-O-methylembelin, berberine, (-)-(S)-equol, alpha-linolenic acid, polidocanol, juglone, etc. The bioassay-guided fractionation, compounds isolation from an active fraction of methanol extract, and their anticancer activity and molecular mechanism study *in vitro* and *in vivo* could be important future studies.

Abbreviations

MNBM	Methanol extract of *M.napaulensis* bark
TPC	Total phenolic content
TFC	Total flavonoid content
GAE	Gallic acid equivalent
QE	Quercetin equivalent
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ZOI	Zone of inhibition
MIC	Minimal inhibitory concentration
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
PBS	Phosphate buffer saline
SCDM	Soyabean Casein Digest Medium
SDA	Sabouraud Dextrose Agar
DMEM	Dulbecco's Modified Eagle Medium
ATCC	American Type Culture Collection
ECACC	European Collection of Authenticated Cell Cultures
A549	Human lung cancer cell line
HeLa	Human cervical cancer cell line
LC–MS/MS	Liquid chromatography-tandem mass spectrometry

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Authors' contributions

RKS, a PhD student in Biotechnology Department, Kathmandu University, Nepal and the first author, was involved in the whole research works, analysis of results, and manuscript preparation. SN, who assisted in the anticancer lab work, is a PhD student in Cancer Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi, India. PSM and BP are MTech students and supported in the lab works. GDJ helped in the analysis of LC–MS/MS results. Both NSB and RPS guided and supervised the anticancer research work and edited the final manuscript. BGS, a PhD supervisor of RKS, designed the research work, supervised, and edited the final manuscript. All authors read and approved the final manuscript and are accountable for its integrity.

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Data availability

Raw and processed LC–MS/MS data from this study can be retrieved from the online repository at the Indian Metabolome Data Archive (IMDA) of the Indian Biological Data Centre (IBDC) (https://ibdc.dbtindia.gov.in/imda/home.html) with IBDC Project accession no. IMP_100015 and study accession no. IMS_100011. A plant herbarium specimen was deposited at National Herbarium and Plant Laboratories (KATH), Godawari-3, Lalitpur, Nepal and the voucher specimen number is KATH167089.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Verbal/email consent was taken from the researchers/co-authors involved in the project for publication.

Competing interests

The authors declare no competing interests.

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