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# In-vitro antioxidant, anti-cancer, and anti-inflammatory activities of selected medicinal plants from western Nepal

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## Abstract

**Background:** This study was focused on the measurement of anticancer properties of six medicinal plants from western Nepal in three cell lines; HeLa, Hep3B, and HCT116, and anti-inflammatory properties in RAW 264.7 cell line through NO, PGE<sub>2</sub>, and TNF- $\alpha$  production. In addition, the phytochemical screening, total phenolic, flavonoid content, and antioxidant properties were evaluated.

**Results:** The qualitative phytochemical analysis revealed the presence of different secondary metabolite and range of total phenolic and total flavonoid content. The highest antioxidant activities were observed in *Bergenia pacumbis* against both DPPH (IC<sub>50</sub> = 25.97  $\pm$  0.19  $\mu$ g/mL) and ABTS (IC<sub>50</sub> = 14.49  $\pm$  0.40  $\mu$ g/mL). Furthermore, the highest antiproliferative effect against cervical, liver, and colon cancer cell lines were observed in *Melia azedarach* as IC<sub>50</sub> values of 10.50, 5.30, and 1.57  $\mu$ g/mL respectively, while the strongest anti-metastatic potential on liver cancer cell line was found in *Pleurospermum benthamii*. In addition, *P. benthamii* showed the most potent anti-inflammatory effect in RAW264.7 murine macrophage cells.

**Conclusion:** This study provided the evidence for *M. azedarach* and *P. benthamii* to have great anticancer potential and finding builds the enough scientific backgrounds in future to isolate and purify the bioactive compounds for further applications.

**Keywords:** Medicinal plants, Anticancer, Anti-inflammatory, Antioxidant

## Background

The use of plant-based medicines for the treatment of various ailments is practiced from ancient times such as Ayurveda, Unani, and Siddha, and has been part of human culture [1–3]. The knowledge of chemical constituents of plants is desirable for the discovery of therapeutic agents and scientific validation of folkloric remedies [4]. Further, plant-based medication are the basis of the modern pharmaceuticals and search for

novel and functional extracts from medicinal plants are in central attraction in recent years because of the presence of diverse bioactive secondary metabolites such as alkaloids, terpenoids, glycosides, steroids, flavonoids, and phenolic compounds [5]. These bioactive molecules are reported to have several beneficial effects in lowering the risk of diseases caused by reactive oxygen species (ROS) [6] through different mechanisms of action such as scavenging free radicals, quenching ROS, inhibiting oxidative enzymes [7]. It is evident that plant extracts have diverse biochemical activities such as anti-allergic, anti-inflammatory, antioxidant, anti-microbial, anti-fungal, antiviral, and anti-cancer [7, 8]. The continuous screening of medicinal plants for their potential bioactivities is

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gaining attention in pharmaceutical, food industries, and cosmetics [9].

Cancer is a deadly disease which is proliferating rapidly and becomes one of the growing health problems worldwide due to the lack of widespread and comprehensive early detection methods and proper medicine [10–12]. Instead of significant development in biomedical science, cancer remains ambiguous especially from the therapeutic perspective and one of the greatest challenges to mankind [13, 14]. Further, it is one of the common causes of death contributing toward uncontrolled growth and invasion of the abnormal cells which leads to the formation of tumors in the human body [15, 16]. It is evident that natural products and bioactive compounds derived from medicinal plants and herbs have significant pharmacological activities and act as a cancer suppressant [17]. Different epidemiological and pharmacological studies suggested that the daily intake of certain phytochemicals can reduce the incidence of several types of cancer [18, 19]. Furthermore, inflammation and oxidative stress have been reported to play an important role in the development of several diseases [20]. Although, anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs (NSAID) are used for the treatment of inflammatory diseases, suffer from the side effect such as gastro-toxicity, hence it is an utmost need of development of safer anti-inflammatory agents [21].

*Melia azedarach* (Meliaceae) is commonly called as 'Bakaina'; traditionally used to cure skin disease, circulatory system disorder, fever, stomach problems, cuts, cough, aches, and dental problems [22] and have already proven antioxidant, antimicrobial, wound healing, hepatoprotective, anthelmintic, and antipyretic activities [23]. *Asparagus racemosus* (Asparagaceae) is commonly called as 'Kurilo'; traditionally used to cure stomach problems and skin diseases [24] and have antihepatotoxic, antineoplastic, antiprotozoal, adaptogenic, antiulcer, antioxidant, antilithiatic, antidepressant, anti-inflammatory, and cytotoxicity activities [25]. *Thymus linearis* (Lamiaceae) is commonly called as 'Ghodamarcha'; traditionally used to cure stomach problems, cough, cold, and eye infection [26] and have antihypertensive [27], anti-inflammatory, antipyretic [28], hepatoprotective [29], antioxidant, anticholinergic [30], and antimicrobial [31] activities. *Pleurosperrum benthamii* (Apiaceae) is commonly called as 'Gandhaina' and used to cure Diarrhea, dysentery, constipation, gastritis, cough, cold, headache, and indigestion [2]. *Bergenia pacumbis* (Saxifragaceae) is commonly called as 'Pakhanved' and used to cure diarrhea, dysentery, vomiting, fever, cough, kidney problems, cold, urinary problems, and indigestion [32]. *Murraya koenigii* (Rutaceae) is commonly called as 'Kadipatta'; traditionally used to cure skin diseases, stomach problems, body aches, dental problem, and urinary tract infection [33]

and have antimicrobial, antioxidant, anticancer, anti-inflammatory, wound healing, hepatoprotective, antifungal, neuroprotective, and antidiabetic activities [34].

The western region of Nepal is rich in plant diversity of high medicinal values [35]. The increasing use of traditional practices for the treatment of diseases by herbal medicine demands more scientifically sound evidence for the ideologies behind therapies and for the effectiveness of plant-based medicines [36]. The six medicinal plants; *M. azedarach*, *A. racemosus*, *T. linearis*, *P. benthamii*, *B. pacumbis*, and *M. koenigii* from Karnali region of Nepal were selected based on their traditional medicinal uses and most used plant species for the in-vitro evaluation of biochemical activities. The main objectives of this study were to evaluate the total phenolic and flavonoid content; antioxidant properties; anticancer properties in three cell lines, HeLa, Hep3B, and HCT116; and anti-inflammatory properties in RAW 264.7 cell line through NO, PGE<sub>2</sub>, and TNF- $\alpha$  production.

## Methods

### Cell lines

Colon carcinoma (HCT116), hepatocarcinoma (Hep3B), cervical carcinoma (HeLa), and RAW264.7 murine macrophage cell lines were obtained from Korean Cell Line Bank (KCLB).

### Plant collection, authorization, and extract preparation

Plants were collected from the Humla and Mugu District of Karnali Province and Banke district of Province 5 of Nepal. The plant species were scientifically authorized in the National Herbarium and Plant Laboratory (KATH) Godawori, Lalitpur, Nepal (Table 1). The collected plant samples were dried at room temperature and made powdered by grinding in an electrical grinder. Ten-gram powder of each sample was macerated with 100 mL of absolute methanol (HPLC-grade) and kept overnight on a rotary shaker (JEIO TECH, South Korea) at 150 rpm at 25 °C temperature. On the next day, the entire mixture was filtrated and concentrated to dryness by evaporating on a vacuum evaporator (hanil Modul 4080C, South Korea) as the slightly modified procedure of previous investigators [37]. The extracted crude samples were kept in airtight glass vials and stored at 4 °C until use.

### Qualitative phytochemical screening

The qualitative phytochemical analysis of Alkaloids, Saponin, Quinone, Sterol, Cardiac glycoside, Tannin, Terpenoid, Protein, and Reducing sugar was performed as the procedure followed by the previous investigators [38–40].

**Table 1** Detail on plants collection

Plants (Family)	Plant code	Vernacular name	Collection site	Collected parts
<i>Melia azedarach</i> L. (Meliaceae)	T1	Bakaina	28° 12' 12" N 81° 48' 06" E	Leaves
<i>Asparagus racemosus</i> Willd. (Asparagaceae)	T2	Ban Kurilo	28° 12' 12" N 81° 48' 06" E	Rhizome
<i>Thymus linearis</i> Benth. (Lamiaceae)	T3	Ghodamarcha	29° 07' 52" N 82° 30' 50" E	Leaves
<i>Pleurospermum benthamii</i> (DC.) C.B. Clarke (Apiaceae)	T4	Gandhaino	29° 38' 38" N 82° 06' 37" E	Leaves
<i>Bergenia pacumbis</i> (Buch. -Jam.ex D. Don) (Saxifragaceae)	T5	Pakharved	29° 07' 52" N 82° 30' 50" E	Roots
<i>Murraya koenigii</i> (L.) Spreng. (Rutaceae)	T6	Kadipatta	28° 12' 12" N 81° 48' 06" E	Leaves

#### Determination of total flavonoid content

Aluminum chloride (AlCl<sub>3</sub>) colorimetric assay was performed for the determination of total flavonoid content (TFC) as the procedure described by previous investigators with slide modifications [41]. The absorbance of the reaction mixture was measured at 510 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800). In detail, 500 µL plant extract of 1 mg/mL concentration was mixed with 2 mL of distilled water and 150 µL of 5% NaNO<sub>2</sub>. After 5 min of incubation at dark, 150 µL of 10% AlCl<sub>3</sub> was added and incubated further for 6 min. Then, 1 mL NaOH of 1 M concentration and distilled water was added to maintain 5 mL reaction volume and absorbance was taken. TFC was quantified from the calibration curve ( $R^2 = 0.990$ ) with quercetin as a standard. The results were expressed as milligram of quercetin equivalents per gram of dry weight of extract (mg QE/g DW).

#### Determination of total phenolic content

Folin-Ciocalteu's technique was used to determine total phenolic content (TPC) as the procedure of previous investigators with slight modifications [42]. The absorbance was measured at 765 nm using a UV-Visible spectrophotometer. In detail, 500 µL crude plant extract of 1 mg/mL concentration was mixed with 800 µL distilled water and 100 µL Folin-Ciocalteu's reagent. Then, 300 µL of 7% Na<sub>2</sub>CO<sub>3</sub> was added after 5 min of incubation at dark followed by another 30 min of incubation in dark and dilution of reaction mixture to 2 mL by addition of distilled water. TPC was quantified from the calibration curve ( $R^2 = 0.994$ ) with gallic acid as a standard. Results were expressed as milligram of gallic acid equivalent per gram dry weight of extract (mg GAE/g DW).

#### In-vitro DPPH and ABTS assay for the determination of antioxidant activities

Free radical scavenging was determined by 1,1-diphenyl 1-2-picryl-hydrazyl (DPPH) assay [43]. For the measurement of antioxidant activity, 1 mL of plant extracts (10, 20, 40, 60, 80, 100 µg/mL) was mixed with 3 mL of the DPPH solution of 100 mM concentration. The reaction mixtures were incubated for 30 min in dark and absorbance was recorded at 517 nm using a UV-visible spectrophotometer. An equal volume of methanolic DPPH in

the place of the sample was used as a positive control and gallic acid as a standard.

In addition, the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation decolorization assay was also performed for the evaluation of antioxidant potential of the plant extracts by using standard procedures followed by the previous investigators slight modifications [44]. An aliquot of 1 mL of plant extract (10, 20, 40, 60, 80, 100 µg/mL) was mixed with 3 mL of ABTS working solution and incubated for 10 min in dark. The absorbance was taken at 734 nm. The 50% methanolic ABTS in the place of plant extract was used as a positive control and gallic acid as a standard.

The percentage of DPPH and ABTS scavenging was calculated by the following formula.

%Radical scavenging

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} * 100\%$$

#### Anticancer activities

##### Cell culture and growth assay

The anticancer activities of the plant extract were measured in three different cell lines HCT116 (colon carcinoma), Hep3B (hepatocarcinoma), and HeLa (cervical carcinoma). HCT116 colon carcinoma cells were grown in RPMI 1640 medium containing 10% fetal bovine serum (FBS). Whereas, Hep3B hepatocarcinoma and HeLa cervical carcinoma cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and grown at 37 °C in a humidified 5% CO<sub>2</sub> incubator. For cell growth assay, cells seeded at 2 × 10<sup>3</sup> cells/well into 96-well culture plates (SPL Lifesciences, Gyeonggi, Korea) were treated with each plant extract at various concentrations (3.12, 6.25, 12.5, 25, 50, 100, 200 µg/mL) for 72 h. Cell growth was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT) colorimetric assay [45]. The absorbance of each well was determined at a wavelength of 540 nm using a microplate reader (Thermo Fisher Scientific, Finland).

### Wound healing assay

Cell migration was analyzed using a wound healing assay in the Hep3B cell line by using the standard protocol [46]. Briefly, cells were seeded into 24-well plates and grown to 90% confluence. The confluent monolayer cells were scratched using the 10 µL of a sterile pipette tip and washed using PBS buffer to remove the non-adherent cells in each well. The cells were treated with the plant extract at different concentrations (25 and 50 µg/mL) and control cells were untreated with plant extract. The cells were further grown up to 48 h and the perimeter of the central cell free zone was imaged with an optical microscope (Olympus, USA).

### Anti-inflammatory activities

#### Cell culture and cytotoxicity assay

The anti-inflammatory activities of the plant extract were measured in RAW264.7 murine macrophage cells [47]. The cells were grown in DMEM medium supplemented with 10% FBS and maintained at 37 °C in a humidified 5% CO<sub>2</sub> incubator. The RAW264.7 cells (5 × 10<sup>3</sup> cells/well) were seeded into 96-well culture plate with various concentrations of the plant extracts (0.39, 0.78, 1.56, 3.12, 6.25, 12.5 µg/mL) and incubated for 24 h. The cell viability was measured by the addition of 10 µL of WST-1 reagents to each well, and the cells were incubated for 2 h. The absorbance of each well was determined at a wavelength of 450 nm using a microplate reader.

#### Measurement of NO production

The NO production was determined by using Griess reagent [48]. Briefly, the RAW264.7 cells (5 × 10<sup>3</sup> cells/well) were treated with the plant extracts (1, 2.5, 5, 10, 20 µg/mL) for 1 h, followed by incubation with 1 µg/mL LPS for another 24 h. Briefly, conditioned medium (50 µL) was mixed with the same volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride) and then incubated at room temperature for 5 min. The amount of NO production was determined at 540 nm using a microplate reader.

### Measurement of PGE<sub>2</sub> and TNF-α by enzyme-linked immunosorbent assay

Briefly, RAW264.7 cells were treated with the plant extract (1, 2.5, 5, 10 µg/mL) for 1 h and followed by incubation with 1 µg/mL LPS for 24 h. The supernatant was used to determine the production of PGE<sub>2</sub>, TNF-α in RAW264.7 cells using the enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions as by previous investigators [49].

### Statistical tools

All the analyses were performed in triplicate for TFC, TPC, antioxidant, anticancer, and anti-inflammatory activities, and the results were expressed as mean value ± standard deviation. The IC<sub>50</sub> values for antioxidant activities were calculated by using a radical scavenging percentage in Microsoft Excel 2013. In the wound healing, NO, PGE<sub>2</sub>, and TNF-α assays, statistical analysis was analyzed using the analysis of variance (one-way ANOVA) with SPSS statistics package (SPSS 9.0; SPSS Inc.). Post hoc analysis was carried out by Tukey's test. A *p* value of <0.05 was considered a statistically significant difference.

## Results

### Phytochemicals, total flavonoid content, and total phenolic content

The results on qualitative phytochemicals analysis revealed the presence of diverse secondary metabolites namely alkaloids, saponin, quinone, sterol, cardiac glycoside, tannin, terpenoid, protein, and reducing sugar in most of the plant extracts (Table 2). Among all, alkaloids, cardiac glycoside, and terpenoid were highly detected phytochemicals, whereas saponin was the least. The extract of *B. pacumbis*, *T. linearis*, and *A. racemosus* showed high phytochemicals content while least by *M. azedarach*.

The results obtained from a preliminary analysis of total flavonoid content (TFC) and total phenolic content (TPC) were summarized in Table 3. The highest TFC was found in *B. pacumbis* (145.70 ± 0.38 mg QE/g DW) followed by *M. koenigii* (102.68 ± 1.38 mg QE/g DW)

**Table 2** Results on qualitative phytochemical screening

Plants	Alkaloids	Saponin	Quinone	Sterol	Cardiac glycoside	Tannin	Terpenoid	Protein	Reducing sugar
<i>M. azedarach</i>	+	-	-	-	+	-	+	-	-
<i>A. racemosus</i>	+	+	+	+	+	-	+	+	+
<i>T. linearis</i>	+	-	+	+	+	+	+	+	+
<i>P. benthamii</i>	+	-	+	+	+	-	+	+	-
<i>B. pacumbis</i>	+	-	+	+	+	+	+	+	+
<i>M. koenigii</i>	+	-	-	+	+	+	+	-	+

-; Not detected, +; Detected

**Table 3** Results on total flavonoid content and total phenolic content

Plants	Total flavonoid content (mg QE/g DW)	Total phenolic content (mg GAE/g DW)
<i>M. azedarach</i>	20.31 ± 0.83	96.02 ± 0.89
<i>A. racemosus</i>	3.61 ± 0.59	12.55 ± 0.60
<i>T. linearis</i>	61.22 ± 0.06	78.20 ± 1.32
<i>P. benthamii</i>	6.86 ± 0.77	11.38 ± 0.94
<i>B. pacumbis</i>	145.70 ± 0.38	208.39 ± 0.65
<i>M. koenigii</i>	102.68 ± 1.38	178.04 ± 1.12

and *T. linearis* (61.22 ± 0.06 mg QE/g DW). Whereas, least TFC was observed in *A. racemosus* (3.61 ± 0.59 mg QE/g DW). Similarly, the highest TPC was found in *B. pacumbis* (208.39 ± 0.65 mg GAE/g DW) followed by *M. koenigii* (178.04 ± 1.12 mg GAE/g DW) and *M. azedarach* (96.02 ± 0.89 mg GAE/g DW). The least phenolic content was observed in *A. racemosus* (12.55 ± 0.60 mg GAE/g DW) and *P. benthamii* (11.38 ± 0.94 mg GAE/g DW).

**Antioxidant activities**

The overall results on IC<sub>50</sub> values for DPPH assay and ABTS assay were determined on the basis of radical scavenging percentage and are shown in Table 4. Our results revealed the highest antioxidant activities in *B. pacumbis* against both DPPH (IC<sub>50</sub> = 25.97 ± 0.19 µg/mL) and ABTS (IC<sub>50</sub> = 14.49 ± 0.40 µg/mL) followed by *T. linearis* against DPPH (IC<sub>50</sub> = 142.59 ± 2.60 µg/mL) and ABTS (IC<sub>50</sub> = 64.92 ± 1.68 µg/mL) and least was found in *A. racemosus* against both DPPH (IC<sub>50</sub> = 873.80 ± 49.10 µg/mL) and ABTS (IC<sub>50</sub> = 502.72 ± 9.80 µg/mL). The percentage of radical scavenging of plant extracts was shown in Fig. 1.

**Anticancer activities**

To evaluate the anticancer activities of the plant extracts, we examined the anti-proliferative effect of plant extract against HeLa, Hep3B, and HCT116 cancer cell lines. Analyzed plant extracts revealed a dose-dependent anti-proliferative effect. The cancer cells were cultured for 72

h in the presence of each plant extract at various concentrations, and then viable cells were measured by MTT assay. Among six extracts tested, *M. azedarach* most effectively inhibited the growth of HeLa, Hep3B, and HCT116 cancer cells, with IC<sub>50</sub> values of 10.50, 5.30, and 1.57 µg/mL, respectively (Fig. 2). Followed by *M. koenigii* and *P. benthamii* extract with IC<sub>50</sub> value of 43.82, 31.38, and 42.73 and 42.00, 81.15, and 31.63 µg/mL respectively toward HeLa, Hep3B, and HCT116 cell line (Table 4).

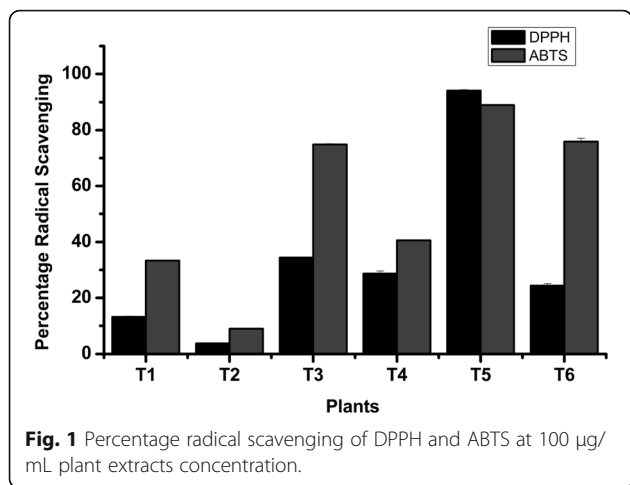
In order to evaluate the effectiveness of the plant extracts on metastasis of cancer cells, it was analyzed using the wound healing assay against Hep3B cell line. Anti-metastatic effect of the plant extract against the Hep3B was analyzed and the percentage migration was recorded at 24 h and 48 h of incubation. Results revealed that plant extract has tendencies to suppress the migration of the cell. The inhibitory effects of the plant extracts on the Hep3B liver cancer cell migration were evaluated. The result showed that the wound scratch in untreated control cells was fully closed after 48 h of incubation, whereas treatment with plant extracts suppressed effect on the migration of Hep3B cells (Fig. 3a, b).

**Anti-inflammatory activities**

To determine the effects of the plant extracts on RAW264.7 macrophage cell viability, the WST-1 assay was performed at 24 h after treatment with the indicated concentrations of the plant extracts. Five other plant extracts, except *M. azedarach*, did not cause any significant cytotoxicity up to 12.5 µg/mL in RAW264.7 cells (Fig. 4a, b). Furthermore, to evaluate the effects of the plant extracts on production of nitric oxide (NO), a principal pro-inflammatory mediator, RAW264.7 cells were pretreated with the plant extracts for 1 h and then stimulated with lipopolysaccharide (LPS) for 24 h. Our results revealed that the extract of *P. benthamii* shows the significant inhibition in LPS-induced NO production as compared with other LPS-treated plant extracts with no cytotoxicity. To our knowledge, this is the first report of the inflammatory activities of the *P. benthamii*. In order to further confirm the anti-inflammatory activities

**Table 4** Results on antioxidant and anticancer activities

Plants	IC <sub>50</sub> values for antioxidant activities (µg/mL)		IC <sub>50</sub> values for antiproliferative activities (µg/mL)		
	DPPH	ABTS	HeLa	Hep3B	HCT116
<i>M. azedarach</i>	424.62 ± 12.22	151.57 ± 1.22	10.50 ± 1.50	5.30 ± 1.36	1.57 ± 1.33
<i>A. racemosus</i>	873.80 ± 49.10	502.72 ± 9.80	> 200.00 ± 1.16	136.2 ± 1.06	133.20 ± 1.06
<i>T. linearis</i>	142.59 ± 2.60	64.92 ± 1.68	162.3 ± 1.03	81.56 ± 1.11	113.60 ± 2.17
<i>P. benthamii</i>	173.30 ± 2.69	122.39 ± 0.84	42.71 ± 1.60	81.15 ± 1.11	31.63 ± 1.15
<i>B. pacumbis</i>	25.97 ± 0.19	14.49 ± 0.40	49.00 ± 1.10	157.1 ± 1.33	> 200.00 ± 1.49
<i>M. koenigii</i>	194.65 ± 4.41	58.74 ± 0.32	43.82 ± 1.09	31.38 ± 1.22	42.73 ± 1.06



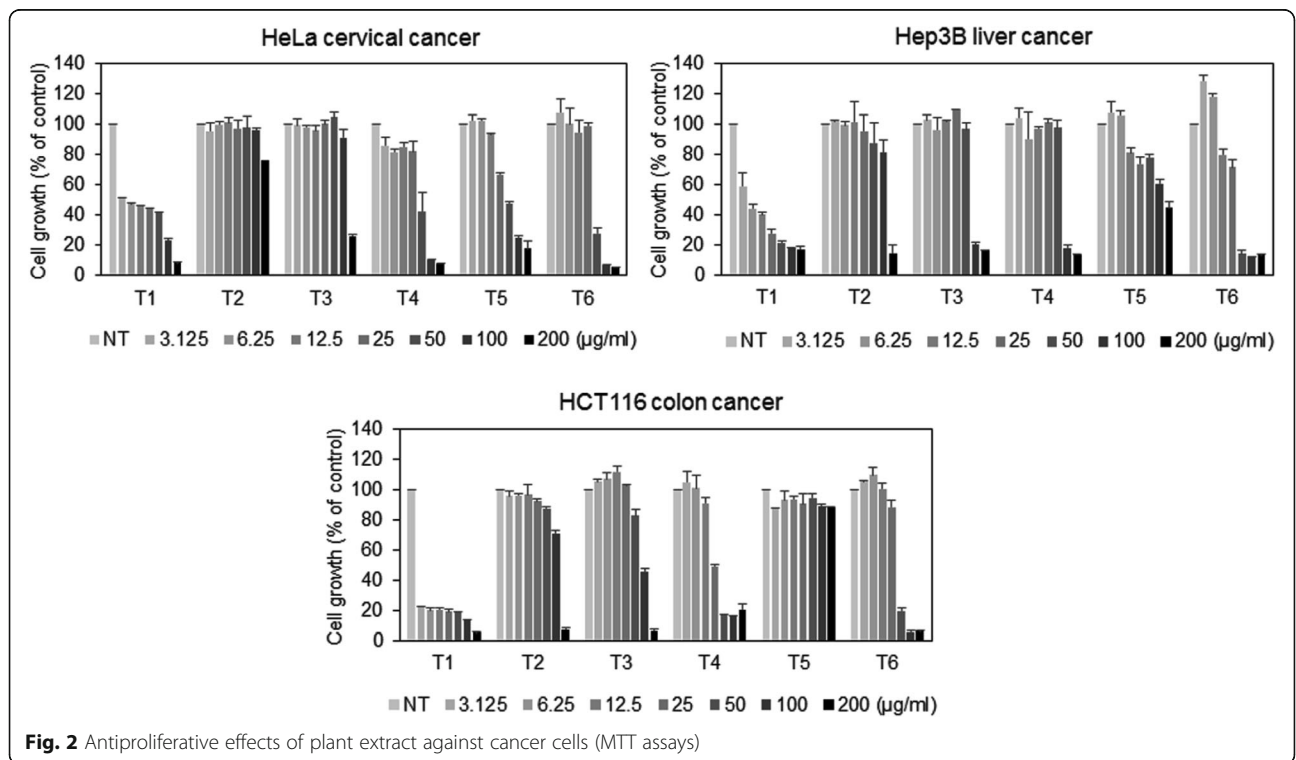
of the *P. benthamii* methanol extract, we next evaluated the other pro-inflammatory cytokines PGE<sub>2</sub> and TNF-α in the culture supernatant that were determined by ELISA. Our results revealed that *P. benthamii* methanol extract reduced the LPS-induced expression of the PGE<sub>2</sub> and TNF-α in dose-dependent manner (Fig. 4c, d).

**Discussion**

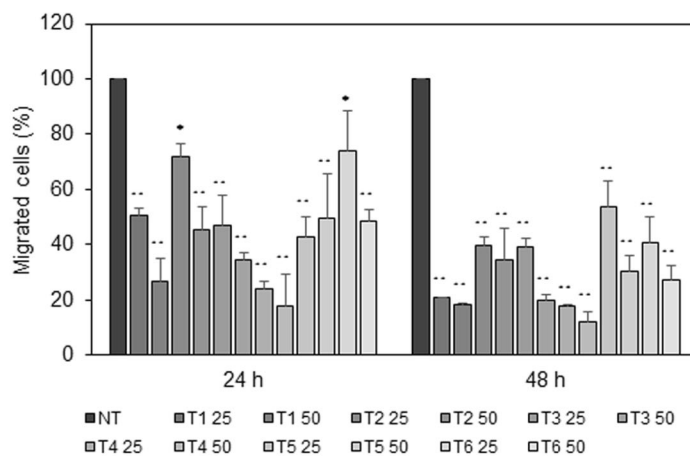
In developing countries, around 70–80% of the population relies on medicinal herbs and plants as primary health care [50]. Although modern drugs are accessible and effective in curing many diseases, herbal remedies are gaining popularity day by day owing to its fewer side

effects and toxicity [51]. In our present study, we evaluated the medicinal plant extract collected from the Western region of Nepal for their potential health benefits such as antioxidant, anti-cancer, and anti-inflammatory activities. Crude methanol extract revealed the presence of diverse phytonutrients such as steroids, flavonoids, phenolic compounds, terpenoids, and alkaloids. Although, flavonoids and phenolic compounds are well-known for their high antioxidant activities and associated with several pharmaceutical and medicinal applications reflected by its anti-cancer effect, cardioprotective effects, immune system promoting, and anti-inflammatory effects [52]; however, the correlation between the chemicals constituents and their bioactivity are not always positive. In recent years, plant-based polyphenols and flavonoids are used as the substitute for the many artificial antioxidants. It is evident that natural antioxidants are safer to use compared to artificial antioxidant, which is suspected to have adverse health effects [53, 54]. Among six medicinal plants extract analyzed, *B. pacumbis* and *M. koenigii* have shown the highest TPC and TFC, whereas *A. racemosus* have shown the least amount. In order to further evaluate the biological activities of these extracts, we next investigated the antioxidant, anticancer, and anti-inflammatory activities.

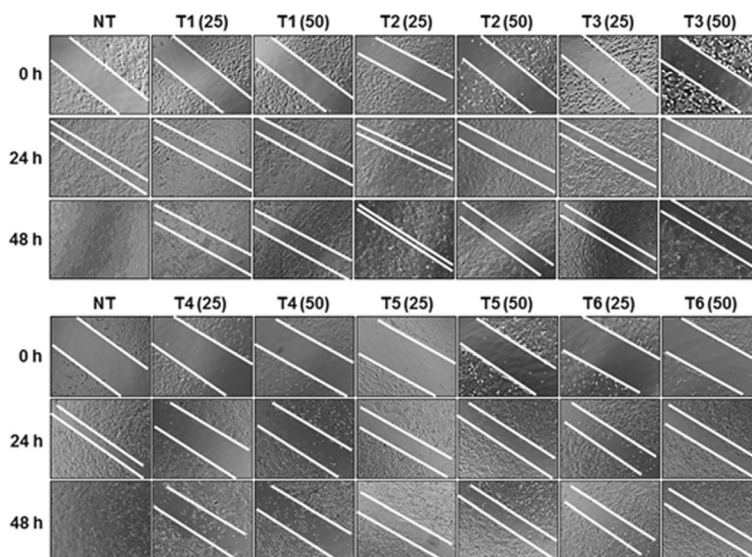
Furthermore, chemotherapy is widely used for the treatment of cancer; its long-term exposure has several other health complications. Hence, scientists are trying to identify alternative treatment with fewer side effects. It is evident that the secondary metabolites from plants such as



**Fig. 2** Antiproliferative effects of plant extract against cancer cells (MTT assays)



(A)

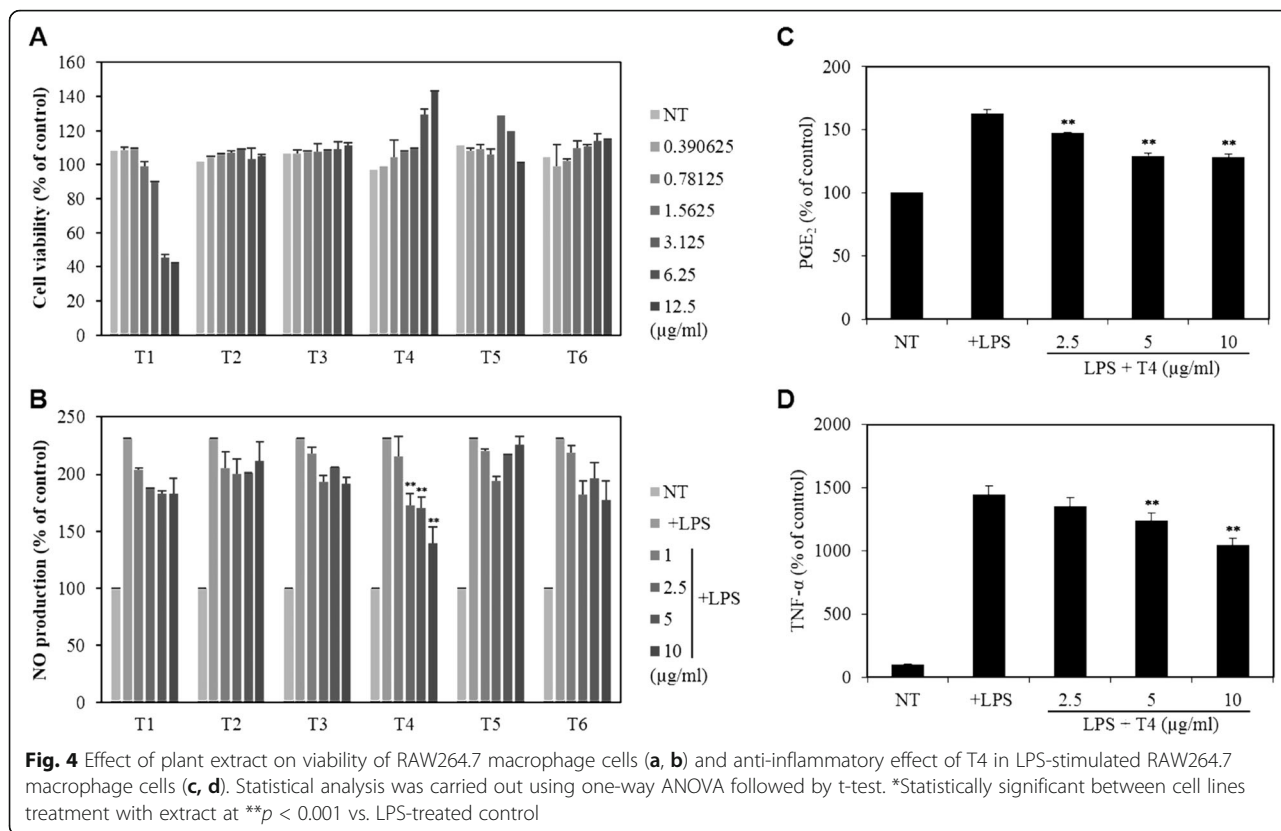


(B)

**Fig. 3** Antimetastatic effects of plant extract against Hep3B cells (a, b). Statistical analysis was carried out using one-way ANOVA followed by t test. \*Statistically significant between cell lines treatment with extract at  $p < 0.05$ ,  $p < 0.001$  vs. the control

terpenoids, phenolics, and alkaloids have a broad spectrum of bioactivities including anticancer properties [55]. However, their use in clinical practice is not possible due to their toxicity and imperfect bioavailability. Thus, modification of the chemical structure of secondary metabolites from plants is one strategic way to increase their anticancer action and decrease their toxicity and side effects [56, 57]. Hence, continuous screening of the medicinal herbs and plants for its potential anticancer activities is of growing scientific interest. Our analysis revealed the anti-cancer potential of *M. azedarach*, *P. benthamii*, *B. pacumbis*, and *M. koenigii* extracts against HeLa, Hep3B, and HCT116 cell lines among six analyzed plant extract. However, *A. racemosus* and *T. linearis* showed relatively lower inhibitory activities against the analyzed cancer cell lines and also found toxic to the normal cell.

Although studies on anticancer potential of different fractions and pure isolates of *M. azedarach*, *A. racemosus*, *T. linearis*, and *M. koenigii* have been already carried out in different cell lines, no detail investigations were carried out for these plant extracts against examined cancer cell lines. This is the first report on anticancer and anti-inflammatory activities of *P. benthamii* and *B. pacumbis*. Previous studies on *M. azedarach* extract against human urinary bladder carcinoma cells and human breast cancer cells possess no significant inhibition [58, 59]. However, some steroids isolated from *M. azedarach* showed good anticancer potential with low cytotoxicity against human lung cancer cell lines and the human glioma cancer cell line [60]. The essential oil from *T. linearis* showed prominent anti-proliferative activity against human breast cancer MCF-7, hormone-



dependent prostate carcinoma, and fibroblast cell lines [61]. Our result on *T. linearis* is comparable with the previous work on antiproliferative effects on the HCT-116 cell line [62]. In particular, Thymol is a marker compound of Genus *Thymus* and possesses the great anticancer activities on different cell lines but possesses cytotoxicity against HeLa cell in a concentration-dependent manner [63, 64] and intestinal cells line [65]. Similarly, Shatavarin IV from *A. racemosus* possesses significant anticancer activity against human breast cancer, human colon adenocarcinoma, and human kidney carcinoma cell lines [66]; silver nanoparticles from *A. racemosus* shows cytotoxicity effect against ovarian cancer cell line [67]; and crude root extract shows cytotoxicity against lung cancer cells [68]. A preliminary screening of leaf extracts of *M. koenigii* exhibited significant anticarcinogenic effects on inhibiting the growth of breast cancer cell line [69] and girinimbine (a carbazole alkaloid) isolated from *M. koenigii* shows good antiproliferative effects against lung cancer cells [70]. Moreover, *M. koenigii* leaf extract possesses a dose-dependent decrease in cell viability/proliferation of human colon, prostate, liver, and cervical cancer cell lines [71]. Although numerous researches showed potential anticancer properties of these plant extracts, the activity based on pure isolates must be done for the authentication and scrutiny of absolute anticancer potential and feasibility of drug formulation from the plant extracts.

The plant-based medicine induces wound healing and regeneration of the lost tissue by multiple mechanisms which are also considered cheap, affordable, and safe. However, scientific validation, standardization, and safety evaluation of plants used in traditional medicine should be carried out before we recommend those plant materials for further application [72]. This is the first report on the wound healing capacity of *P. benthamii* and *B. pacumbis*. Our study revealed the wound healing activity of *P. benthamii* and *B. pacumbis* methanol extract and has great tendencies to suppress the migration of Hep3B cells. It is also less toxic compared to the other plant extract examined. Our finding opens up the possibilities in the future to identify the potential anticancer compounds in *P. benthamii* and *B. pacumbis*. Flavonoids and phenolics are among the most potent ingredients that underlie the protective effect of diets rich in fruits, vegetables, and plants with respect to minimize the risk of causing cancer [73, 74]. Hence, flavonoid compounds could probably be responsible for the anticancer activity of the plant extracts [69]. However, more research should be carried out for the understanding of the correlation between flavonoids and anticancer activities of plant extracts.

Inflammation is a physiological response to tissue injury, which is triggered by the physical, toxic chemical, and microbial agents. The inflammatory process involves



acute and chronic. The nitric oxide (NO) is a well-known pro-inflammatory mediator associated with much physiological process. Hence, the NO production was used as tools for the measurement of the inflammation; reduction of NO production has been characterized as an effective strategy for the treatment of inflammation-related diseases. Furthermore, transcription factors such as NF- $\kappa$ B are responsible for the expression of the pro-inflammatory mediators during the process of inflammation. The NF- $\kappa$ B pathway is triggered by pro-inflammatory stimuli such as IL-1 and TNF- $\alpha$ . The primary mediators of acute inflammation include histamine, serotonin, and COX-2, whereas chronic is mediated by PGE<sub>2</sub>, NO, and lipoxigenases [75]. It is reported that chronic inflammation might lead to ailments such as peptic ulcer, rheumatoid arthritis, and cancer as well [76]. Our result revealed that among six medicinal plants extract evaluated, *P. benthamii* showed potential anti-inflammatory activities with no toxicity against the RAW264.7 murine macrophage cells. Our results revealed that analyzed plant extracts have cytotoxicity and wound healing results; we analyzed only *P. benthamii* for anti-inflammatory properties because of its less toxicity and good activity. There are several examples of other plant species having anti-inflammatory properties. Taking into the account  $\beta$ -carboline alkaloids, C-1 and C-2, isolated from *M. azedarach* have a potential therapeutic effect on inhibiting NO production [77], the treatment of murine macrophages with *A. racemosus* significantly inhibited OTA-induced suppression of chemotactic activity and production of TNF- $\alpha$  [78], Girinimbine from *M. koenigii* inhibits the release of NO in RAW 264.7 cells [79], and different *Thymus* species [80–82] and *Bergenia* species [83] have already known anti-inflammatory properties.

## Conclusion

Among the six selected plant species evaluated for its potential biological activities, *M. azedarach* revealed the good anti-cancer. However, *P. benthamii* shows remarkable wound healing and anti-inflammatory activities with no toxicity. Although, other plant species possess anti-proliferative activity, the toxicity was high for the consideration of anticancer therapeutic. Our result opens up the possibility in the future to identify the potential therapeutic agents from *P. benthamii* for the development of herbal-based medicine.

## Abbreviations

ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DMEM: Dulbecco's modified eagle medium; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FBS: Fetal bovine serum; GAE: Gallic acid equivalent; HCT116: Colon carcinoma; HeLa: Cervical carcinoma; Hep3B: Hepatocarcinoma; KCLB: Korean Cell Line Bank; LPS: Lipopolysaccharide; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide; QUE: Quercetin equivalent; TFC: Total flavonoid content; TPC: Total phenolic content

## Acknowledgements

We would like to thank Sun Moon University, South Korea for providing facilities for anticancer and anti-inflammatory activities analysis and we would also like to thank National Herbarium and Plant Laboratory (KATH), Godhawori, Lalitpur Nepal and Prof. Dr. Ram Prasad Chaudhary for the plant identification and authorization.

## Study involving plants

The plant species were scientifically authorized in the National Herbarium and Plant Laboratories, Department of plant resources, Ministry of forest and soil conservation, Government of Nepal with reference (Ref No. 2074/075-230).

## Authors' contributions

BPP conceptualized the work. KA and SPP perform the photochemical analysis. TPC, TFC, and antioxidant measurements. BPP, HJS, EKL, and HJJ perform the anti-cancer and anti-inflammatory activities analysis and data interpretation. SPP, BPP, and HJS wrote, edited, and revised the manuscripts. All authors have read and approved the final manuscript.

## Funding

Funded by H-Plant Pvt. Ltd. through the grant support (2012-2017) from Korea International Cooperation Agency (KOICA) and partial support through grant support from NRF Basic Science Research Program (NRF-2016R1D1A1B03932956) and NRF grant funded by the Ministry of Science and ICT (NRF-2019R1A2C1009033).

## Availability of data and materials

Data will be available by corresponding authors upon reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

Authors declare no any conflict of interest.

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Received: 26 November 2019 Accepted: 7 September 2020

Published online: 29 October 2020

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