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Phytochemical and Pharmacological Comparison of Wild and Micropropagated Methanolic Leaf Extracts of *Ulmus wallichiana*

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This study compared the phytochemicals, antioxidant, antibacterial, anti-inflammatory and cytotoxic activities of wild (WUWE) and micropropagated (MUWE) *Ulmus wallichiana* methanolic leaf extracts. Direct shoot regeneration was successfully optimised using DKW medium along with 8.8 μmol/L BAP and 5 μmol/L PCIB, resulting in high shoot induction (85.65 ± 0.20%), shoot number (7.46 ± 0.22) and shoot length (5.35 ± 0.23 cm). Phytochemical analysis revealed that MUWE contained higher levels of alkaloids, flavonoids, phenols and carbohydrates than WUWE. Antibacterial screening showed effective inhibition by both extracts against *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis*, with MUWE exhibiting slightly superior activity. Antioxidant potential, assessed using DPPH and ABTS assays, indicated stronger free radical scavenging by MUWE (IC_{50} 23.81 and 20.66 μg/mL) compared to WUWE (IC_{50} 30.72 and 29.83 μg/mL). MUWE also displayed higher anti-inflammatory activity (IC_{50} 26.77 μg/mL) than WUWE (IC_{50} 35.89 μg/mL). The cytotoxicity against HCT-116 colon cancer cells revealed enhanced antiproliferative activity by MUWE (IC_{50} 75.24 μg/mL). Phase contrast microscopy confirmed these effects, showing cell rounding, fragmentation and loss of confluence with MUWE treatment. These findings suggest that micropropagation enhances both the phytochemical richness and therapeutic efficacy of *U. wallichiana*.

Keywords: *Ulmus wallichiana*, Micropropagation, Phytochemicals, Antioxidant activity, Cytotoxicity, Antibacterial activity.

INTRODUCTION

Medicinal plants have been used throughout the centuries due to their potential in treating many diseases. Medicinal plants contain different phytoconstituents known as secondary metabolites, which includes flavonoids, alkaloids, phenols and tannins [1,2]. Due to the presence of these secondary metabolites, they exhibit multiple therapeutic applications which includes antibacterial, antioxidant, anticancer, anti-inflammatory activities [3,4]. Over the years, there has been increased demand for medicinal plants as they are safe, contains natural compounds and are less toxic than to synthetic drugs, which often have side-effects [5,6].

Ulmus wallichiana Planch, commonly known as Kashmir elm or Bhutan elm, is a deciduous tree found in India, Nepal, Bhutan as well as in the Gilgit-Baltistan region of Pakistan and Nuristan region of Afghanistan [7-9]. It possesses tremendous demulcent, emollient, astringent, expectorant and diuretic properties. Moreover, this plant has significant commercial and therapeutic potential and have been extensively studied for their effects on osteoporosis and osteoclastogenesis [10]. The bark of this plasssnt is used for wound healing and its leaves were utilised as fodder to sheep and goats particularly in the regions of Jammu & Kashmir [11]. *U. wallichiana* contains aliphatic hydrocarbons, triterpenes and some alleopathic compounds like alnulin, betulin, catechol, lupenol and vanilin [12]. In some studies, it has been found the bark of *U. wallichaina* contains ulmoside A, B and naringenin-6-C-β-glucopyranoside [13]. The plant possesses immense pharmaceutical and medicinal properties which includes antibacterial, antioxidant, anti-hypersensitive, anti-inflammatory, anticancer, cardioprotective and neuroprotective [7]. Although the leaves of *U. wallichiana* are primarily valued for ornamental foliage, their phytochemical and pharmacological potential remains insufficiently investigated. Considering that leaves often serve as rich sources of bioactive secondary metabolites, the limited number of studies on *U. wallichiana* leaves represents a significant gap in the existing literature [14]. However, comprehensive evaluations of methanolic leaf extracts, including those concerning their anticancer properties, remain scarce. To the best of our knowledge, this is the first report evaluating the biological activities of WUWE and MUWE methanolic leaf extract, thereby highlighting the novelty and significance of our study.

Advancements in plant tissue culture have facilitated the *in vitro* propagation of medicinal plants, offering a sustainable approach to producing bioactive compounds under controlled conditions. *In vitro* culture techniques including callus induction and organogenesis, enable the mass propagation of medicinal plants while maintaining their genetic and phytochemical integrity [15]. Comparative evaluations between *in vitro* and wild-derived extracts can provide insights into the consistency and efficacy of secondary metabolite production. This study was aimed to compare phytochemical profile, antioxidant, antibacterial, anti-inflammatory and anticancer properties of methnolic leaf extracts of *U. wallichiana* (WUWE and MUWE).

EXPERIMENTAL

Explant selection and the sterilisation treatment: The *U. wallichiana* plant material was collected from the Kashmir University Botanical Garden (KUBG), located at coordinates (34º07′57′′N, 74º50′15′′E) and identified by Akhter Ahmad Malik Curator and Plant Taxonomist, University of Kashmir. A voucher specimen (Voucher No.9876-KASH) has been deposited in the KASH Herbarium, Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir, Srinagar, Jammu & Kashmir, India. Nodal explants obtained from *U. wallichiana* were used for micropropagation studies. The length of these explants was trimmed to 0.3 to 0.5 cm and then placed under the running tap water for about 0.5 h to remove the unwanted dust. The explants were then soaked in detergent "Labolene"1 % (v/v) with 2-3 drops of surfactant "Tween 20" and then subjected to washing to remove the detergent. Further, the explants were treated with 10% of bevistin (w/v) (methyl-3-benzimidizole carbonate) solution to avoid the fungal contamination. The explants were sterilised by 70% ethanol and 0.01 % (w/v) $AgNO_3$ solution and washed thoroughly with double distilled water. Using sterile surgical blade, the explant was carefully cut before being inoculated onto the precooled autoclaved media.

Culture media and plant growth hormones for shooting and rooting: To micropropagate *U. wallichiana* DKW (Driver and Kuniyuki Walnut) full basal media was used with different concentrations and combinations of cytokinin and anti-auxin. The plant growth regulator BAP with concentration ranging from (2.2-11.1 μmol/L) and anti-auxin PCIB (*p-para* chloro phenoxy isobutyric acid with concentration of (2-10 μmol/L) were employed. The explants were inoculated on the culture media and the media was switched every 15-20 days in order to promote the shoot and root growth.

Plant extract: The WUWE and the MUWE leaves were used for the extraction. Both the plant samples were washed under the running tap water, dried in a shade for about 10 days and placed in the brown paper bags and kept in a cool place for several days before making the powder. The dried plant material was grounded by using motor and pestle, subsequently, 20 g of each (WUWE and MUWE) plant material was soaked in 200 mL of methanol. The mixtures were placed on an orbital shaker for about 72 h at 23-25 ºC. The plant materials were then subjected to the cold extraction at the room temperature for 24 h. The filtrate was collected using Whatman No. 1 filter paper and kept refrigerated until analysis.

Qualitative phytochemical analysis: *U. wallichiana* wild (WUWE) and micropropagated (MUWE) methanolic leaf extracts were subjected to phytochemical analysis using standard protocols [16].

Test for alkaloids: A 1 mL of leaf extract was mixed with 2-3 drops of Wagner reagent. The presence of yellow colour indicates the evidence of alkaloids.

Test for flavonoids: A 2 mL of leaf extract was added with small pieces of magnesium ribbon followed by addition of dropwise 5% of sulphuric acid. After few minutes, the formation of pink colour confirms the presence of flavonoids.

Test for terpenoids: A 1 mL of leaf extract was mixed with 1 mL of chloroform followed by the addition of few drops of sulphuric acid in a tube and then mixed vigorously. The formation of yellow colour at the bottom of tube indicates the presence of terpenoids.

Test for tannins: To a 10% of $FeCl_3$ solution, the leaf extract was mixed vigorously. Formation of blue green colour confirmed the presence of tannins.

Test for phenols: In a test tube, 1 mL of leaf extract was mixed with 2-3 drops of $FeCl_3$. The gradual change in colour observed after a short incubation period indicates the presence of phenols.

Test for carbohydrates: In a test tube, 2 mL of leaf extract was added with Benedict's reagent. The formation of red orange precipitate indicates the presence of carbohydrates.

Test for saponins: 1 mL of leaf extract was diluted with 5 mL of distilled water. After shaking the mixture for 15 min, stable foam formed in the tube, indicating the presence of saponins.

Test for proteins: To detect the presence of proteins in the sample 2 mL of extract was mixed with 2 mL of HNO_3.

Test for glycosides: To 10 mL of leaf extract, 4 mL of glacial acetic acid was added followed by the addition of 2% of $FeCl_3$. The formation of brown rings between layers indicates the presence of glycosides.

Quantitative phytochemical analysis: The total flavonoid content of WUWE and MUWE samples was evaluated using the acid-base precipitation method. The extracts were treated with acetic acid in ethanol and precipitated using ammonium hydroxide. The resulting precipitate was collected, dried and weighed and the alkaloid content was expressed as grams per 100 g of dry extract.

The total phenolic content of WUWE and MUWE samples was evaluated using Folin-Ciocalteu (FC) method. The extracts were reacted with FC reagent and $NaCO_3$, incubated for 30 min in dark and absorbance was measured at 765 nm. The results were expressed as grams of gallic acid equivalents (GAE) per 100 g of dry extract.

Antibacterial assay: The antibacterial activity of WUWE and MUWE methanolic leaf extracts of *U. wallichiana* methanolic extracts were carried out using well diffusion assay. The petri-plates were added with sterile Muller-Hinton Agar (MHA) until it solidifies. The test pathogens at a concentration of 10^4 to 10^6 cfu/ mL were swabbed using sterile cotton swab. On the surface of each petri-plate the wells were made using 1 mL tip. The extracts at the concentration ranging from (20-80 µg/mL) from the stock solution of 10 mg/mL were inoculated into the respective wells for 24 h at 37 ºC. DMSO was used as negative control and ampilox and levofloxacin were used as positive controls respectively. The zone of inhibition was measured in mm.

Antioxidant activity

DPPH assay: The antioxidant activity of *U. wallichiana* WUWE and MUWE methanolic extracts was measured using the DPPH assay, which was slightly modified from the method developed by Arika *et al.* [17]. The leaf extracts of both MUWE and WUWE of *U. wallichiana* were used and ascorbic was used as standard in varying concentrations starting from 10-60 µg/mL. A DPPH (1 mM) were prepared in methanol and subsequently were added in diluted concentrations of extracts and standard in sterile test tubes and shake for some time in order to mix well and kept in dark sconditions at room temperature for 30 min. In a similar manner, methanol and DPPH were used to prepare a blank solution without extract. Using a blank as a reference, the spectrophotometer measured the sample absorbance at 517 nm. Each experiment was conducted three times. ± SD was used to indicate the findings of the calculation of the percentage of inhibition. The percentage of scavenging activity was determined by using the following formula:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

ABTS assay: ABTS scavenging assay of *U. wallichiana* methanolic extracts of both WUWE and MUWE was carried out to neutralize $ABTS^+$ radicals by using reported method with slight modifications [18]. In order to prepare ABTS radical cation ($ABTS^+$) 7 mM of ABTS was added with 2.45 mM potassium persulphate and kept in dark for 12-16 h in ambient temperature. Later on, ABTS solution was diluted with methanol in order to reach its absorbance up to 0.700, at 734 nm. Both methanolic extracts of WUWE and MUWE ranging from 10-60 µg/mL were added with the diluted $ABTS^+$ solution. The mixture was stirred for 0.5 h and later the absorbance was measured at 734 nm. The percentage of inhibition was calculated by using the following formula:

$$\text{ABTS}^+ \text{ neutralizing effect (\%)} = \frac{AB - AA}{AB} \times 100$$

where AB = the absorbance of $ABTS^+$ mixed with methanol and AA = the absorbance of the $ABTS^+$ added with the sample or standard. The experiment was carried out in triplicates and ascorbic acid was used as standard control.

Anti-inflammatory activity: The anti-inflammatory activity of diclofenac (standard), WUWE and MUWE was evaluated using the protein denaturation inhibition assay. Stock solutions (10 mg/mL) of each sample were prepared and diluted to concentrations ranging from 10 to 60 µg/mL. A 0.5 mL of 1% BSA, 0.5 mL of the test sample and 1 mL of PBS (pH 6.4) were mixed. The mixtures were incubated at 37 ºC for 20 min and then heated at 70 ºC for 5 min to produce protein denaturation. The absorbance at 660 nm was measured after cooling and the % inhibition was calculated. Every test was run three times. The following formula was used to determine the percentage of inhibition:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

In vitro cytotoxicity assay

Cell culture: The HCT-116 Cell was procured from National Centre for cell Science (NCCS), Pune, India. The cells were cultured in DMEM media supplemented with 10% FBS and 100U/mL of penicillin and 100 µg/mL of streptomycin. The culture was maintained in humidified incubator with 5% CO_2 at 37 ºC.

Cell viability assay: MTT assay was used for *in vitro* cytotoxic activity of WUWE and MUWE samples [19]. Around 6×10^3 HCT-116 cells were seeded per well. The cells were exposed to different concentrations ranging from (20-100 µg/mL) of methanolic leaf extracts of both WUWE and MUWE and then incubated for 24 h with 5% CO_2 at 37 ºC. Subsequently, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated at 37 ºC for 4 h. Untreated cells served as the control. After incubation, the medium was discarded and 100 µL of DMSO was added to dissolve the formazan crystals, followed by gentle shaking. Finally, absorbance was recorded at 590 nm using a microplate reader and cell viability (%) was calculated using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Statistical analysis: Experiments were performed in triplicate and the data were statistically analysed using Graph Pad Prism version 9.0.0 with one-way ANOVA followed by Tukey's post hoc test and Duncan's multiple range test. Data are expressed as mean ± SD (n = 3); significance was analysed by two-way ANOVA followed by Tukey's test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant).

RESULTS AND DISCUSSION

***In vitro* micropropagation of *U. wallichiana*:** The leaves of *U. wallichiana* were grown in DKW medium as shown in Fig. 1 with varying concentrations of BAP and PCIB in µmol/L. BAP was used from the concentration ranging from 2.2-11.1 µmol/L and PCIB 2-10 µmol/L as shown in Table-1.

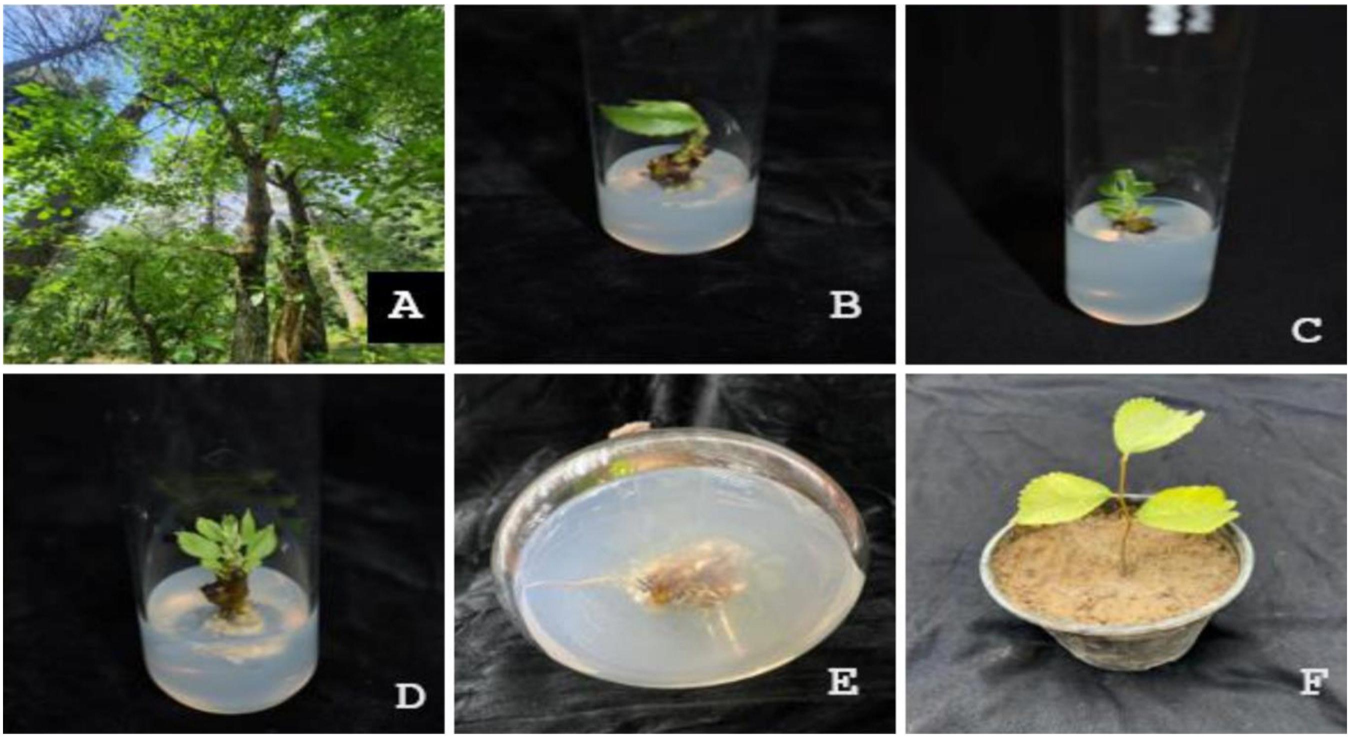

Fig. 1. Direct micropropagation of *Ulmus wallichiana* (A) habitat; (B) shoot initiation from nodal explant; (C&D) shoot development; (E) root initiation; (F) acclimatised plantlet

TABLE-1
EFFECT OF DKW MEDIA AND DIFFERENT CONCENTRATIONS AND COMBINATIONS OF PLANT GROWTH REGULATORS ON SHOOT INDUCTION FROM NODAL EXPLANT

PGR's (µmol/L)		Nodal explant			
BAP	PCIB	Percentage response (mean ± SD)	Mean no. of shoots (mean ± SD)	Average no. of days (mean ± SD)	Average shoot length (cm) (mean ± SD)
2.2	–	36.95 ± 0.43^{u}	1.56 ± 0.21^{k}	25.86 ± 0.32^{a}	2.56 ± 0.18^{g}
	2	53.78 ± 0.30^{t}	3.56 ± 0.26^{h}	21.75 ± 0.25^{f}	3.71 ± 0.25^{cde}
	5	62.81 ± 0.30^{n}	5.51 ± 0.24^{e}	18.88 ± 0.21^{jkl}	3.98 ± 0.88^{bcde}
	10	60.51 ± 0.24^{q}	4.73 ± 0.24^{fg}	20.76 ± 0.26^{g}	3.48 ± 0.26^{def}
4.4	–	37.68 ± 0.22^{u}	1.50 ± 0.27^{k}	23.70 ± 0.22^{c}	2.78 ± 0.14^{gf}
	2	58.91 ± 0.50r	4.75 ± 0.14^{fg}	20.50 ± 0.28^{gh}	3.50 ± 0.24^{de}
	5	68.88 ± 0.19^{k}	5.73 ± 0.25^{de}	19.50 ± 0.25^{ijk}	4.51 ± 0.24^{b}
	10	67.63 ± 0.21^{l}	4.55 ± 0.27^{g}	21.66 ± 0.22^{f}	4.18 ± 0.20^{bcd}
6.6	–	35.66 ± 0.22^{v}	2.48 ± 0.27^{i}	24.51 ± 0.28^{b}	2.61 ± 0.20^{g}
	2	60.65 ± 0.2^{q}	5.48 ± 0.26^{ef}	20.38 ± 0.24^{gh}	3.43 ± 0.24^{def}
	5	71.66 ± 0.30^{h}	6.41 ± 0.26^{cd}	18.81 ± 0.13^{kl}	3.48 ± 0.23^{def}
	10	70.03 ± 0.11^{i}	5.56 ± 0.23^{e}	19.78 ± 0.14^{hi}	3.41 ± 0.22^{ef}
8.8	–	34.58 ± 0.30^{w}	2.58 ± 0.22^{i}	24.55 ± 0.28^{b}	2.68 ± 0.13^{g}
	2	73.55 ± 0.29^{g}	6.35 ± 0.21^{cd}	20.51 ± 0.24^{g}	4.56 ± 0.20^{b}
	5	85.65 ± 0.20^{c}	7.46 ± 0.22^{b}	16.58 ± 0.23^{n}	5.35 ± 0.23^{a}
	10	84.00 ± 0.41^{d}	7.00 ± 0.86^{bc}	18.48 ± 0.27^{l}	4.65 ± 0.22^{b}
11.1	–	30.46 ± 0.27^{x}	2.58 ± 0.27^{i}	26.53 ± 0.28^{a}	2.55 ± 0.23^{g}
	2	59.05 ± 0.21^{r}	5.45 ± 0.24^{ef}	20.55 ± 0.23^{g}	3.55 ± 0.22^{de}
	5	62.60 ± 0.23^{o}	6.45 ± 0.20^{cd}	19.61 ± 0.17^{ij}	4.55 ± 0.20^{b}
	10	60.00 ± 0.28^{q}	5.18 ± 0.23^{efg}	21.46 ± 0.23^{f}	3.56 ± 0.22^{de}

*Each value represents the mean ± standard deviation. Values followed by different letters in their superscript are significantly different from each other ($p < 0.05$) according to Duncan's multiple range test

The highest shoot induction (85.65 ± 0.20%), maximum shoots (7.46 ± 0.22), shortest days (16.58 ± 0.23) and longest shoot length (5.35 ± 0.23 cm) were observed at 8.8 µmol/L BAP + 5 µmol/L PCIB. The lowest response (30.46 ± 0.27%) and shoots (2.58 ± 0.27) were observed at 11.1 µmol/L BAP alone. Therefore, BAP + PCIB combinations improved shoot induction. A study conducted by Shukla *et al.* [16] on *Ulmus americana* showed that maintaining low levels of hormones resulting in steady growth over longer time. Moreover, appropriate hormone levels were responsible to keep the cultures healthy and productive.

Preliminary phytochemical analysis: Both wild and micropropagated leaves of *U. wallichiana* plant were subjected to preliminary phytochemical analysis to screen for the presence of various bioactive constituents like alkaloids, flavonoids, terpenoids, tannins, carbohydrates, saponins, phenols, proteins and glycosides. as shown in Table-2. The micropropagated leaf extract (MUWE) showed the highest amounts of alkaloids, flavonoids, phenols and carbohydrates, with moderate amounts of tannins, saponins, glycosides, terpenoids and proteins. In contrast, the wild leaf extract (WUWE) exhibited relatively lower levels of alkaloids, flavonoids, proteins and carbohydrates and only trace amounts of phenols, terpenoids, glycosides, tannins and saponins. Thus, MUWE showed higher proportions of these secondary metabolites in the methanolic extract compared to WUWE extract. These results suggest that *in vitro* propagation may positively influence the accumulation of key secondary metabolites, potentially enhancing the medicinal value of the plant. According to Verpoorte *et al.* [17] the *in vitro* conditions may act as an important elicitor for stimulating the production of secondary metabolites due to growth regulators, optimised nutrient media and stress factors [17].

TABLE-2
PRELIMINARY PHYTOCHEMICAL ANALYSIS OF BOTH WUWE AND MUWE METHANOLIC EXTRACTS

Phytochemicals	WUWE	MUWE
Alkaloids	+++	++
Proteins	++	+
Terpenoids	++	+
Glycosides	++	+
Phenols	+++	++
Carbohydrates	+++	++
Saponins	++	+
Tannins	++	+
Flavonoids	+++	++

+++; strongly present, ++; moderately present, +; weakly present

The quantitative analysis revealed significant differences in phytochemical composition between WUWE and MUWE leaf samples. The total phenolic content was higher in MUWE (0.18 ± 0.01 g/100 g) compared to WUWE (0.15 ± 0.01 g/100 g). Similarly, the total flavonoid content was also markedly increased in MUWE (0.42 ± 0.01 g/100 g) relative to WUWE (0.35 ± 0.01 g/100 g) as shown in Fig. 2. These results suggest enhanced biosynthesis of phenolics and flavonoids under controlled *in vitro* culture conditions. The elevated phenolic content in MUWE is expected to contribute positively to antioxidant and related biological activities, as supported by multiple studies demonstrating strong correlations between phenolic levels and bioactivities. For example, in a comprehensive evaluation of medicinal plants from Mizoram, high total phenolic contents were associated with significant antioxidant capacity and antimicrobial effects, indicating that phenolics play a central role in modulating biological efficacy [18]. Similarly, correlation studies across diverse Indonesian herbs revealed that total phenolic and flavonoid contents were

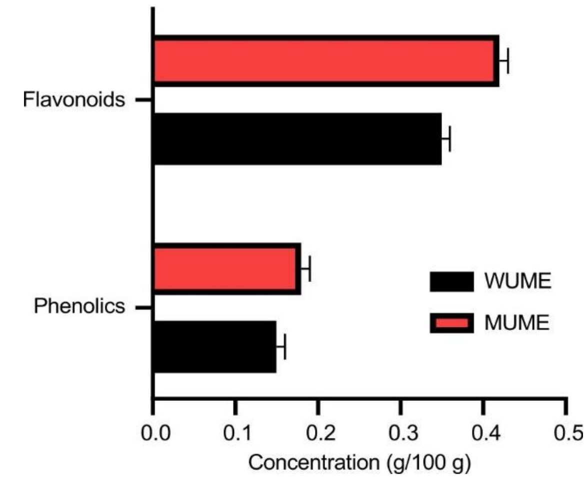

Fig. 2. Comparison of total phenolic and flavonoid contents (g/100 g) in WUWE and MUWE extracts, expressed as mean ± SD

significant contributors to antioxidant activity, reinforcing the impact of polyphenols on free radical scavenging potential [19].

Antibacterial activity: The antibacterial activity of *U. wallichiana* methanolic leaf extracts of both WUWE and MUWE were tested against four different pathogens, with *Enterococcus Faecalis* and *Staphylococcus aureus* being Gram-positive and *Escherichia coli* and *Pseudomonas aeruginosa* being Gram-negative in a dose-dependent manner as shown in Fig. 3a-b. The results suggest that both WUWE and MUWE extracts had good antibacterial activity, however MUWE extract had a higher antibacterial activity than WUWE extract as shown in Table-3. The wild extracts showed comparatively lower antibacterial activity than micropropagated extracts. The results of the present study are inconsistent with the study done by Haque *et al.* [20], who demonstrated higher antimicrobial activity in *in vitro* derived plant extracts compared to wild extracts due to the accumulation higher amounts of secondary metabolites.

Antioxidant activity

DPPH assay: The antioxidant activity of *U. wallichiana* methanolic leaf extracts of both WUWE and MUWE were conducted using the DPPH assay. Different concentrations of WUWE and MUWE methanolic leaf extracts ranging from 10-60 μg/mL were used with ascorbic acid used as standard control. As from the results MUWE showed higher antioxidant activity than WUWE at all the concentrations. At 60 μg/mL MUWE showed maximum scavenging activity around 84.2 ± 1.2% while as WUWE showed maximum radical scavenging around 74.7 ± 1.3% at the same concentration. Ascorbic acid (standard) on the other hand showed maximum radical scavenging activity around 90.3 ± 1.1% which is higher than both the extracts as shown in Fig. 4a. The IC_{50} values were calculated. For MUWE, it was found to be 23.81 μg/mL and for WUWE, it was 30.72 μg/mL, which was higher than MUWE. In comparison to ascorbic acid, it was found to be very low 15.15 μg/mL. The results confirmed that the micropropagated extract MUWE showed significant stronger antioxidant activity compared to WUWE extract. A similar study was conducted by Shanthamma & Nagesh [21], who reported that *in vitro* derived leaf extracts of *Mollugo nudicaulis* also

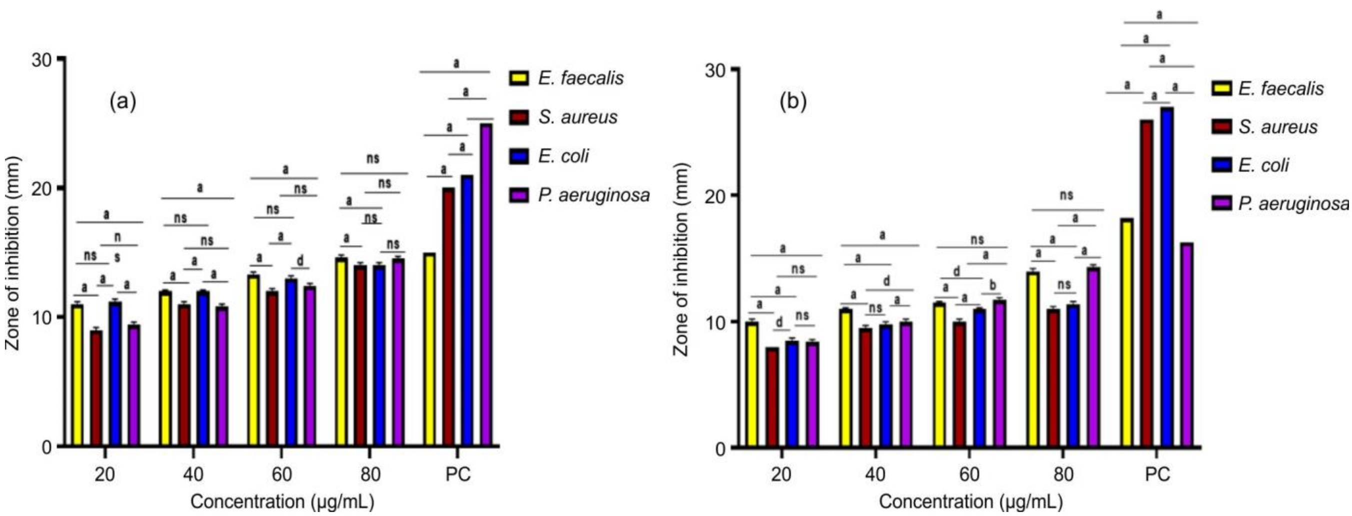

Fig. 3. (a) Zone of inhibition of MUWE against selected bacterial strains at 20-80 µg/mL with ciprofloxacin as positive control; (b) Zone of inhibition of WUWE against the same strains at identical concentrations

TABLE-3
ANTIBACTERIAL ACTIVITY OF MICROPROPAGATED (MUWE) AND WILD (WUWE) METHANOLIC LEAF EXTRACTS OF *U. wallichiana* AGAINST SELECTED BACTERIAL STRAINS

Concentration (µg/mL)	Zone of inhibition (mm)							
	E. faecalis		*S. aureus*		*E. coli*		*P. aeruginosa*	
	MUWE	WUWE	MUWE	WUWE	MUWE	WUWE	MUWE	WUWE
20	11.0 ± 0.3	10.8 ± 0.3	9.0 ± 0.4	8.2 ± 0.2	11.2 ± 0.3	9.1 ± 0.2	9.3 ± 0.3	9.0 ± 0.2
40	12.2 ± 0.2	11.4 ± 0.2	11.0 ± 0.3	9.7 ± 0.2	12.0 ± 0.2	9.8 ± 0.2	11.0 ± 0.3	10.0 ± 0.3
60	13.5 ± 0.3	11.8 ± 0.3	12.0 ± 0.4	11.5 ± 0.3	13.2 ± 0.2	10.5 ± 0.2	12.5 ± 0.3	11.7 ± 0.2
80	14.5 ± 0.4	13.9 ± 0.3	14.0 ± 0.3	11.1 ± 0.3	14.3 ± 0.3	11.0 ± 0.2	14.5 ± 0.2	13.8 ± 0.2
Positive control	15.0 ± 0.2	17.4 ± 0.4	20.0 ± 0.3	25.4 ± 0.5	21.0 ± 0.4	26.1 ± 0.4	25.0 ± 0.3	25.6 ± 0.4

Values are expressed as mean ± SD (n = 3).

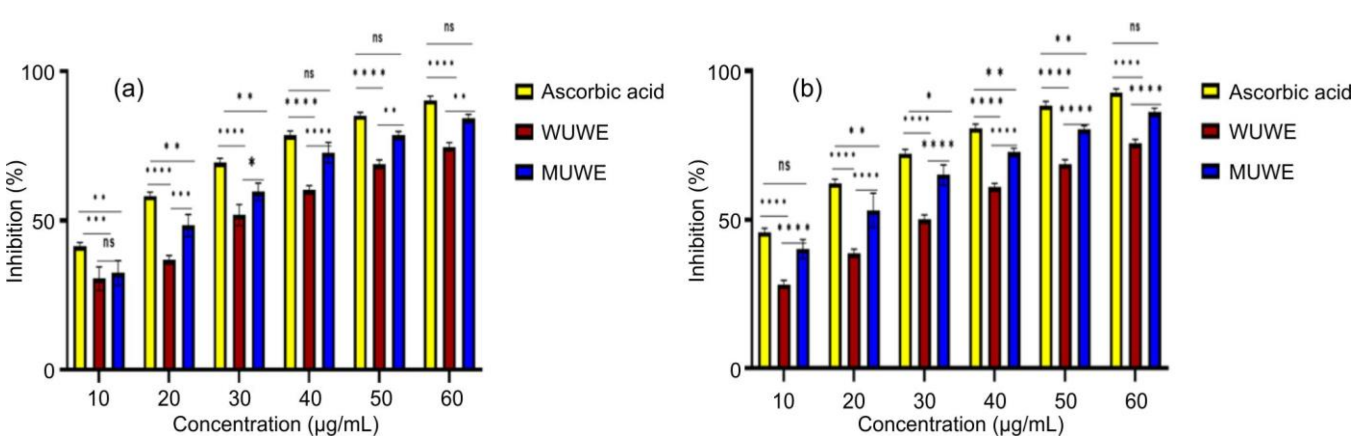

Fig. 4. (a) DPPH radical scavenging activity of WUWE and MUWE compared with ascorbic acid, showing dose-dependent antioxidant activity; (b) ABTS radical scavenging activity of WUWE and MUWE at 10-60 µg/mL with ascorbic acid as positive control

displayed higher antioxidant activity than extracts from naturally grown plants. Similarly, in the current work on *U. wallichiana*, the stronger antioxidant activity of MUWE could be linked to its higher accumulation of phenolic compounds and flavonoids, as indicated by the preliminary phytochemical screening.

ABTS assay: The ABTS radical scavenging activity of the WUWE and MUWE methanolic leaf extracts was carried out on dose dependent manner spanning from 10-60 µg/mL with ascorbic acid as standard control as shown in Fig. 4b. It is evident from the results that MUWE extract displayed more scavenging activity of 86.1 ± 1.2% at 60 µg/mL with IC_{50} value of around 20.66 µg/mL, while in case of WUWE its 75.6 ± 1.2% at the same concentration with IC_{50} value of 29.83 µg/mL. The standard control ascorbic acid (standard) showed maximum scavenging activity of 92.6 ± 1.1% at 60 µg/mL respectively with IC_{50} value of 12.58 µg/mL. The deposition of higher amount of secondary metabolites in MUWE could be linked to higher radical scavenging activity. A high degree of radical scavenging potential could be linked with the comparative higher deposition of various secondary metabolites in the leaf tissues of the micropropagated plants of *M. acuminata* [22].

Anti-inflammatory activity: The leaf methanolic extracts of both MUME and WUWE samples were subjected to anti-inflammatory activity using protein denaturation assay with diclofenac used as standard. On dose dependent manner from (10-60 µg/mL) diclofenac exhibited highest percentage of inhibition 100.0 ± 0.0% at 60 µg/mL. On the other hand, the MUWE extract showed maximum percent of inhibition 84.6 ± 1.2% followed by wild extract WUWE with lower percentage of inhibition 69.3 ± 1.1% at the same concentration. The respective IC_{50} values for diclofenac, MUWE and WUWE were 24.01 µg/mL, 26.77 µg/mL and 35.89 µg/mL, respectively indicating MUWE exhibited significantly higher anti-inflammatory activity than WUWE extract (Fig. 5). A similar study carried out on *Malaxis acuminate* demonstrated that methanolic stem and leaf extacts of micropropagated plants showed notably enhanced anti-inflammatory activity compared to wild extracts [23].

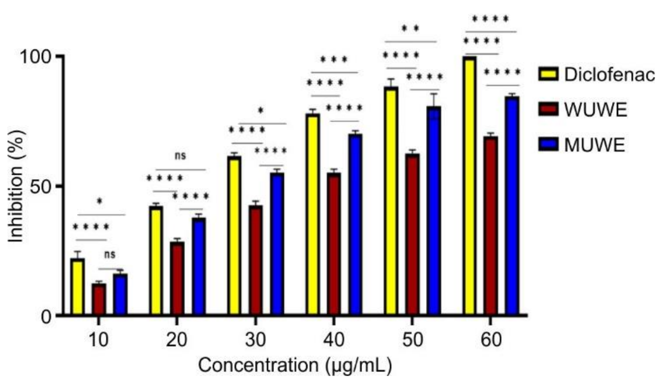

Fig. 5. Anti-inflammatory activity of WUWE and MUWE compared with diclofenac using the protein denaturation assay at 10-60 µg/mL

Cytotoxicity activity: The cytotoxicity effects of WUWE and MUWE methanolic extracts of *U. wallichiana* were carried out using MT assay on dose dependent manner as shown in Fig. 6. It was found both WUWE and MUWE methanolic extracts showed good cytotoxic effects on HCT-116 cancer cells for 24 h. When tested at lower concentration starting from 20 µg/mL the extract of WUWE showed 88% cell viability and MUWE showed 85% respectively. The WUWE showed highest cell viability around 41% at 100 µg/mL, while as MUWE showed 35% at 100 µg/mL respectively. The IC_{50} values of WUWE and MUWE were found to be ≈ 84.54 µg/mL and ≈ 75.24 µg/mL, respectively. The results showed decrease in cell viability were significant ($p < 0.05$) across all the concentrations. According to the results, both extracts demonstrated good antiproliferative activity against HCT-116 cancer cell line, with MUWE exhibiting somewhat higher cytotoxicity than WUWE. The results are in consistent with study carried out on *Scutellaria havanensis* raised by micropropagation where flavonoids and phenolics act as free radical scavengers to induce apoptosis in HCT-116 colon cancer cell line [24]. The enhanced biological activities observed in MUWE can be mechanistically linked to its higher phytochemical content, particularly phenolics and flavonoids, compared to the WUWE. These compounds are well

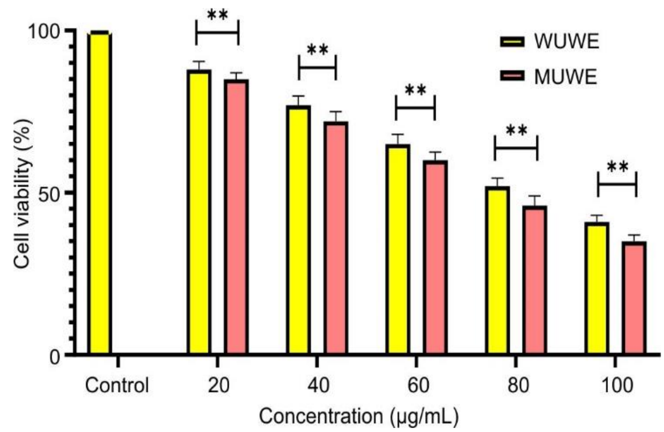

Fig. 6. MTT assay shows percentage of cell viability of HCT-119 treated with different concentrations (20-100 µg/mL) of WUWE and MUWE methanolic extracts for 24 h. On dose dependent manner there is decrease in cell viability. Each experiment was carried out in triplicates and the data were presented as the mean ± standard deviation. $^{**}p < 0.05$

known for their antioxidant properties, which likely contribute to the cytotoxic effects observed against HCT-116 colon cancer cells. Phenolics and flavonoids scavenge reactive oxygen species (ROS), disturbing redox homeostasis in cancer cells, leading to mitochondrial dysfunction, DNA damage and activation of intrinsic apoptotic pathways, as reflected in our phase contrast microscopy observations of cell rounding, fragmentation and loss of confluence [25,26]. In parallel, anti-inflammatory mechanisms involve suppression of NF-κB signalling, which decreases transcription of TNFα and IL-6, while activation of the Nrf2 pathway enhances endogenous antioxidant defenses [27]. The cross-talk between Nrf2 and NF-κB is particularly important, as oxidative stress amplifies inflammatory signalling and chronic inflammation further elevates ROS production, creating a vicious cycle [28]. Thus, compounds with dual antioxidant and anti-inflammatory properties can disrupt this cycle, providing cytotoxic effects against malignant cells while protecting normal tissues.

Morphological analysis: The phase contrast microscopy revealed the significant alterations in HCT-116 colon cancer cells when treated with *U. wallichiana* methanolic extracts. As shown in Fig. 7a, the control cells look healthy and densely packed. The cells show moderate shrinkage and detachment (Fig. 7b) when treated with WUWE at IC_{50} value of ~84.54 µg/mL. In contrast to MUWE at IC_{50} (~75.24 µg/mL), the cells exhibited pronounced rounding, reduced confluence and fragmentation (Fig. 7c) indicating strong cytotoxic effects.

Conclusion

The present study successfully demonstrated efficient *in vitro* micropropagation of *Ulmus wallichiana* using DKW medium supplemented with optimal concentrations of BAP and PCIB, achieving maximum shoot induction and growth at 8.8 µmol/L BAP + 5 µmol/L PCIB. Preliminary phytochemical screening revealed that micropropagated methanolic leaf extract (MUWE) contained a higher concentration of key secondary metabolites compared to wild methanolic leaf extract (WUWE), suggesting enhanced phytochemical accumulation through *in vitro* culture. Further quantitative phytochemical

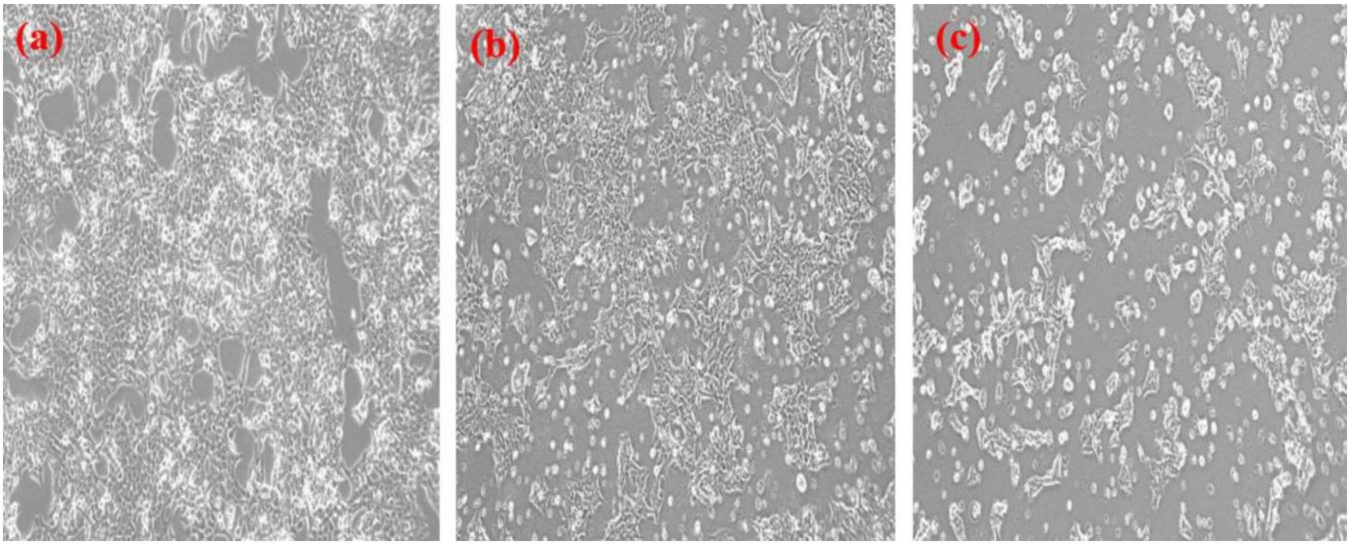

Fig. 7. Morphological changes in HCT-119 colon cancer cells after treated with *U. wallichiana* methanolic extracts of both wild (WUWE) and micropropagated (MUWE) when observed under phase-contrast-microscope; (a) control cells; (b) cells treated with WUWE extract at $IC_{50} \approx 84.54$ µg/mL; (c) cells treated with MUWE at $IC_{50} \approx 75.24$ µg/mL

analysis indicates that micropropagated *U. wallichiana* leaves (MUWE) have higher levels of major secondary metabolites, particularly phenolics and flavonoids, than wild leaves (WUWE), suggesting enhanced biosynthesis under *in vitro* conditions that may contribute to improved antioxidant and biological activities. Bioactivity evaluations showed that both WUWE and MUWE possessed antibacterial, antioxidant, anti-inflammatory and anticancer properties, with MUWE consistently exhibiting superior efficacy. MUWE demonstrated stronger radical scavenging activity in both DPPH and ABTS assays, lower IC_{50} values in anti-inflammatory and cytotoxicity assays and induced more pronounced morphological changes in HCT-116 colon cancer cells compared to WUWE. These findings indicate that micropropagation not only enables sustainable propagation of *U. wallichiana* but may also enhance its therapeutic potential by improving its phytochemical and pharmacological profile. This highlights the promise of micropropagated *U. wallichiana* as a potent source of natural compounds for future pharmaceutical and biomedical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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