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Analysis of phenolic contents and biological activities of wild mint, *Mentha longifolia* (L.) L.

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In alternative and complementary medicine, plant-based remedies have prominent roles. Here, we analysed the wild mint, *Mentha longifolia* (L.) L. collected from Mangesh/Duhok (Iraq) for its phenolic and essential oil content, total antioxidant status (TAS) and total oxidant status (TOS), DPPH free radical scavenging activity, antibacterial, antiproliferative, and DNA protecting activities. Specifically, a Soxhlet apparatus was used to extract the aerial portions of the plant with methanol as well as dichloromethane. The GC-MS instrument measured the amount of essential oils present. A high-performance liquid chromatography (HPLC) was used to analyse the phenolic composition. A Rel Assay kit was used to determine the TAS and TOS levels. Further, DPPH assay was used to quantify antioxidant capacity; MTT assay for antiproliferative efficacy against A549 cancer cell line; and the ability to shield pBR322 supercoiled DNA was tested.. Standard bacteria and fungus strains were used in an agar dilution assay to measure antimicrobial activity. The results demonstrated the plant's capacity to inhibit cell proliferation, low DPPH activity, and significant antimicrobial activity. The plant was found to have TAS value of 2.860 mmol/L, TOS value of 14.858 µmol/L, and an OSI (oxidative stress index) value of 0.522. At 200 µg/mL, the plant extract exhibited DNA-protective effect. The GC-MS study also found the presence of four different chemicals [1-isopropyl-4-methyl-1,4-cyclohexadiene ($C_{10}H_{16}$), pulegone ($C_{10}H_{16}O$), phenol, 2-methyl-5-(1-methylethyl) ($C_{10}H_{14}O$) and caryophyllene ($C_{15}H_{24}$)]. Further, we identified the following phenolic acids: gallic acid, catechin, cinnamaldehyde, chlorogenic acid, coumaric acid, benzoic acid and hydroxy-benzoic acid.

Keywords: Antimicrobial, Antioxidant, Antiproliferative, DNA protective, Horse mint, Silver mint, Spearmint

Plants are important source of essentials such as food, medicine, cosmetics, clothes and even shelter[1]. Humans have also employed several plants to treat various illnesses[2,3]. Vast literature is available on the biological activities of plants viz. antioxidant, anticancer, antimicrobial, DNA protecting, antitumor, antiallergic, antiaging, hepatoprotective and anti-inflammatory[4-9].

The wild mint, *Mentha longifolia* (L.) L. also known as Silver mint or Spear mint, is an aromatic herbaceous perennial with a lot of variation, and has global presence. The upper surface of the leaves has a green to greyish green colour, while the lower surfaces are white[10]. Blooms might be mauve, purple or white. It reaches peak bloom somewhere between the middle and end of summer. In the present study, we explored the phenolic and essential oil contents as well the biological effects of *M. longifolia*.

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Materials and Methods

Aerial parts of the wild mint *Mentha longifolia* (L.) L. was gathered from Mangesh and Duhok in Iraq and preserved at the Herbarium, Biology Department, Zakho University. Dry powder was made from the plant's aerial portions. Powder samples weighing 30 g were obtained and extracted with methanol (MeOH) at 50°C in a Soxhlet device for around 6 h. The dichloromethane (DCM) extracts went through the same process. The solvents used to create the crude extracts were then evaporated using a rotary evaporator.

Total antioxidant, oxidant status and DPPH tests

The Rel Assay TAS (total antioxidant status) and TOS (total oxidant level) kits were used to measure the total antioxidant and oxidant status of the plant's aerial parts, respectively. TAS and TOS testing were conducted according to the manufacturer's instructions. TAS readings were converted to mmol/L using trolox as the standard. The total oxidant status

(TOS) test's calibrator, hydrogen peroxide, was reported in units of micromoles per litre. The oxidative stress index (OSI) (AU: Arbitrary unit) value was determined by dividing the TOS value with the TAS value and taking the percentage[11].

Stock solutions (0.25, 0.5, 1 and 2 mg/mL) of plant extracts were prepared for DPPH assay. About 50 L of each solution was added to 160 L of 0.039% DPPH. A 30-min incubation period followed. Later, the absorbance was measured at 517 nm. All procedures were carried out at several concentrations[12]. rosmarinic acid (RA) and ascorbic acid (AA)Reference antioxidants included.

Determination of antimicrobial activity

MeOH and DCM plant extracts were tested for their antimicrobial activity against a panel of representative bacteria and fungi using an agar dilution method. Plant extracts were used to make stock solutions with concentrations of 800, 400, 200, 100, 50, 25 and 12.5 µg/mL. Standard bacterial strains, including *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacterbaumannii* ATCC 19606, were cultivated in Muller Hinton Broth. In RPMI 1640 Broth pre-cultured fungal strains included *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, *C. glabrata* ATCC 90030. It was determined that inhibition occurred at the lowest dose that stopped colony development[14].

Antiproliferative activity test

MeOH and DCM extracts of the plant were tested for their capacity to sustain A549 cell growth using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. After 70-80% cell confluence, the cells were dissociated using 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA). This was followed by its placement on plates. It was incubated for 24 h after sowing. Next, the concentrations of the extracts were adjusted to 25, 50, 100 and 200 µg/mL, respectively. They were then seeded into plates and incubated for 24 h. The negative controls were grown in growth medium without FCS. The supernatants were dissolved in it after being incubated for 48 h. After incubating the supernatants at 37°C, a purple precipitate was generated after adding 1 mg/mL MTT. Following removal of the supernatants, the MTT taken up by the cells was mixed with dimethyl sulfoxide (DMSO; Sigma-Aldrich; MO, USA). Plates were then analysed at a 570 nm wavelength using an Epoch spectrophotometer (BioTek Instruments, Winooska, VT)[15].

Determination of DNA Protective Activities

The pBR 322 supercoil DNA assay was used to evaluate the DNA protecting properties of the plant's MeOH and DCM extracts. Plant extracts were used to make stock solutions with concentrations of 25, 50, 100 and 200 µg/mL. In Eppendorf tubes, we put 0.5 µg of plasmid pBR 322 supercoil DNA and then added 10 µL of stock solutions. The solution was given a 10 min incubation at room temperature (25°C) after 10 µL of Fenton's agent (30 mM H_2O_2, 50 µM ascorbic acid and 80 µM $FeCl_3$) was added. The final solution volume was made to be 20 mL. The mixture was then kept at 37°C for 30 min. After that, the DNA was run through an electrophoresis on an ethidium bromide stained 1% agarose gel[16].

Determination of phenolic and chemical contents

The plant's chemical make-up was analysed with a Gas Chromatography Mass Spectrometer (Agilent 19091S-433, 30×250×0.25 m HP-5MS). The carrier gas flow rate was 3 mL/min of helium (He). This 280°C split injection was carried out with precision. The oven was preheated at 50°C for 2 min. Experiments were conducted using heating rates of 15°C/min to 120°C/min for 2 min, 5°C/min to 300°C for 16 min, and the detector temperature at 280°C. Mass spectral data was used to identify compounds from the National Institute of Standards and Technology (NIST) library.

The plant's phenolic components were analysed using HPLC. Fifteen commonly found phenolic compounds were checked for (gallic acid, catechin, epicatechin, cinnamic acid, syringic acid, chlorogenic acid, quercetin, caffeic acid, coumaric acid, benzoic acid, t-phenolic, hesperidin, rosmarinic acid, hydroxybenzoic acid and sinapic acid). For this detection, we relied on a DAD detector. Twenty microliters were selected as the injection volume. The flow rate was set at 0.8 mL/min, and the mobile phase was either A: 3% acetic acid or B: methanol. At 30°C, we separated substances chromatographically using a 250×4.6 mm id (inner diameter) 5 µm Agilent Eclipse XDB-C18 column[17].

Results and Discussion

Antioxidant activity

As a byproduct of their metabolic processes, all living things release oxidant reactive oxygen species

(ROS). Antioxidant defence mechanisms are activated in response to an increase in reactive oxygen species, and they work to reduce the production of oxidant molecules[18]. When the body's antioxidant defences are deficient, oxidative stress sets in. In humans, many illnesses, including cancer, Alzheimer's, Parkinson's, cardiovascular disease, etc., are linked to oxidative stress, and antioxidant supplements are useful for mitigating the same[19]. Plants are important in the determination of supplemental antioxidants[20]. In our study, the antioxidant potential of *M. longifolia* was determined. The TAS, TOS and OSI values of *M. longifolia* were 2.860±0.174, 14.858±0.241 and 0.522±0.023, respectively. Table 1 shows DPPH free radical activity of the methanolic extract of *Mentha longifolia* at different concentrations.

Researchers have found antioxidant activity in *M. longifolia* using a variety of methods[21,22]. Our results have also confirmed the antioxidant activity of the *M. longifolia*, though both the extracts showed comparatively less activity than the standards, ascorbic and rosmarinic acid. DCM extract was relatively more effective. These findings demonstrate that plant *M. longifolia* possesses antioxidant properties. As an maiden attempt, we determined the total antioxidant status (TAS), total oxidant status (TOS) and oxidative stres index (OSI) values of *M. longifolia*. Such studies on different plant species are available: *Mentha longifolia* subsp. *longifolia* (TAS: 3.628, TOS: 4.046, OSI: 0.112), *Allium calocephalum* (TAS: 5.853, TOS: 16.288 & OSI: 0.278), *Scorzonera papposa* (TAS: 6.328, TOS: 11.525 & OSI: 0.182), *Alcea kurdica* (TAS: 3.298, TOS: 8.312 & OSI: 0.252), *Rumex scutatus* (TAS: 8.656, TOS: 4.951 & OSI: 0.057); and *Ferulago platycarpa* (TAS: 5.688, TOS: 15.552 & OSI: 0.273)[23-27]. TAS is a measure of all the antioxidant chemicals present in a given organism[28]. *M. longifolia* has been shown to have a lower TAS value than *M. longifolia* subsp *longifolia*, *A. calocephalum*, *S. papposa*, *A. kurdica*, *R. scutatus* and *F. platycarpa*. Same was the case for total antioxidant status as well. TOS is a measure of all the oxidant chemicals it produces[28]. We have shown that the *M. longifolia* has greater TOS value compared to *M. longifolia* subsp. *longifolia*, *S. papposa*, *A. kurdica*, and *R. scutatus*, but a lower TOS value than *A. calocephalum* and *F. platycarpa.*

The oxidative stress index (OSI) reveals how antioxidant molecules in a given sample prevent oxidant chemicals generated by living organisms[28]. OSI value of *M. longifolia* is more than that of *A. kurdica*, *M. longifolia* subsp. *longifolia*, *S. papposa*, *R. scutatus*, *F. platycarpa* and *A. calocephalum*. Due to its poor antioxidant levels, *M. longifolia* is ineffective in reducing the amounts of oxidant chemicals, and hence, shows high OSI value.

Table 1 — DPPH free radical activity of *Mentha longifolia*

Concentration (mg/mL)	Ascorbic acid (%)	Rosmarinic acid (%)	MeOH*	DCM*
0.25	27.717	25.501	5.807	6.227
0.5	69.398	43.744	12.283	9.474
1	95.721	79.694	22.522	15.606
2	95.740	93.849	25.712	29.417

[*Methanolic and dichloromethan extracts of aerial portion of *M. longifolia*]

Table 2 — Antibacterial and antifungal activities of *Mentha longifolia*

	A	B	C	D	E	F	G	H	J
MeOH	400	400	400	200	200	400	400	200	200
DCM	400	400	400	400	200	200	400	200	200

[A: *S. aureus*, B: *S. aureus* MRSA, C: *E. faecalis*, D: *E. coli*, E: *P. aeruginosa*, F: *A. baumannii*, G: *C. albicans*, H: *C. glabrata*, I: *C. Krusei*. MeOH:vv; DCM:vv. MIC values are presented in units of µg/mL]

Antimicrobial activity

Antibiotics are often used to combat bacterial and viral infections. However, unwitting antibiotic usage has contributed to a steady rise in microbial resistance resulting in the failure of antibiotics to adequately treat infections[29,30]. In this context, the need for novel antibacterial natural resources is important. Hence, here, we studied how *M. longifolia* fared against several common bacteria and fungi. The findings are summarized in Table 2.

In our study, we observed similar activities exhibited by MeOH and DCM extracts of the aerial parts of *M. longifolia*. Both the MeOH and DCM extracts @400 µg/mL were effective against *S. aureus*, *S. aureus* MRSA, *E. faecalis* and *C. glabrata*; and *P. aeruginosa*, *C. albicans* and *C. krusei* @200 µg/mL. MeOH extract was effective at 200 µg/mL and DCM @400 µg/mL However, it was *vice versa* against *A. baumannii*. Earlier studies have also reported *M. longifolia* to be effective against *Aspergillus ochraceus*, ,*A. niger A. flavus*, *Bacillus cereus*, *Candida albicans*, *C. glabrata*, *C. tropicalis*, *E. coli*, *Listeria monocytogenes*, *Micrococcus flavus*, *Penicillium ochrochloron*, *P. funiculosum*, *Pseudomonas aeruginosa* and *S. aureus*[31,32]. Bacterial and fungal strains employed in this investigation confirmed *M. longifolia*'s antibacterial properties. It has potential as a natural antibacterial in this setting.

Antiproliferative activity

Within the realm of alternative medicine, several plant samples are used either as direct therapies or as supplements for cancer. Herbal medicines now make up the vast majority of anticancer treatments[33,34]. Furthermore, current cancer therapies continue to fall short of expectations. In this study, we examined *M. longifolia* to see if it has any antiproliferative effect against A549 cell line. The obtained results are shown in Fig. 1.

Various plant species are known to exhibit antiproliferative properties[35,36]. Here, we found that the antiproliferative effects of *M. longifolia* MeOH extract is minimal. However, at high enough concentrations, DCM extract was highly cytotoxic to A549 cells. DCM extract at 200 µg/mL was shown to have the maximum activity. It reveals that the plant *M. longifolia* can be explored for possible natural molecules with anticancer application.

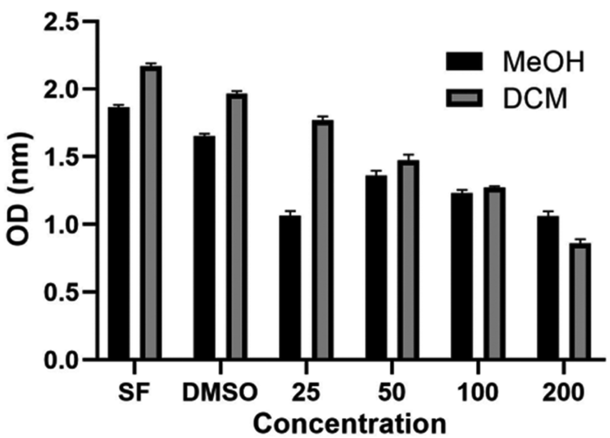

Fig. 1 — Cytotoxic effects of methanolic extract of aerial parts of *Mentha longifolia* [*DMSO: dimethyl sulfoxide; SF: serum-free medium]

DNA protective activity

When antioxidant chemicals are present, DNA is shielded from damage caused by the hydroxyl radical. High quantities of antioxidant chemicals in natural goods have been shown to be useful in protecting against DNA damage. In this context, we examined whether or not *M. longifolia* had a DNA-protective function. The obtained results are shown in Fig. 2.

Our research found that a 200 µg/mL concentration of a MeOH extract of *M. longifolia* has DNA-protective effects. Various authors have indicated in the scientific literature that plants can help keep DNA from becoming damaged[37]. As a result of our research, we found that *M. longifolia* could shield DNA from damage caused by free radicals.

Phenolic and chemical contents

There are chemicals with biological activity that are produced by plants. The pharmacological effect is the result of the presence of bioactive substances[38]. Tables 3 & 4; Fig. 3 show the details of phenolic and other chemical components identified in *M. longifolia* extracts.

Secondary metabolites or bioactive molecules, play an important role in a wide variety of biological processes[39,40]. Phenolic contents were detected from *M. longifolia* extract at different wavelengths using HPLC. Scanned wavelengths are shown in Fig. 3. It shows that the compounds detected in the plant

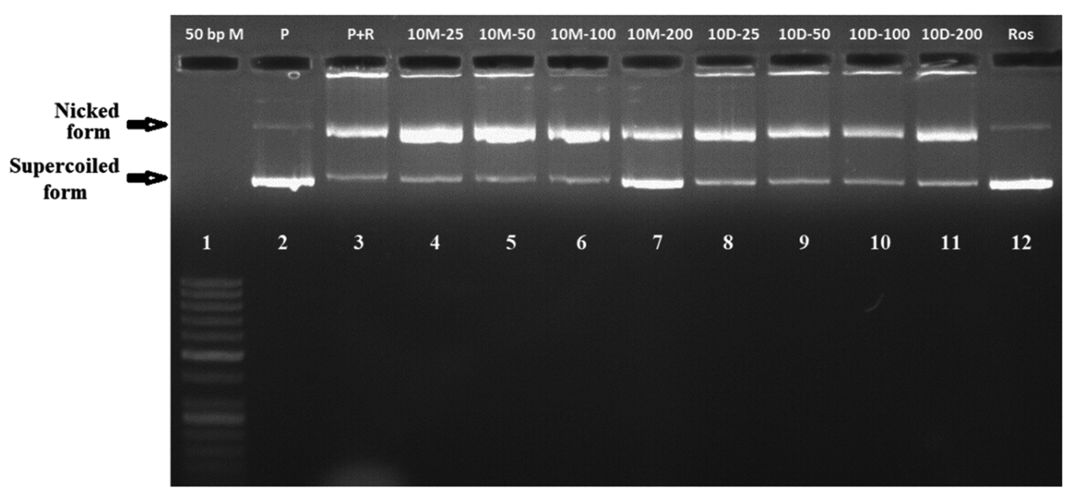

Fig. 2 — DNA protective activity of *Mentha longifolia* extracts [*Lanes: 1, Marker (5 bp); 2, pBR322 DNA; 3, Negative control, pBR322 DNA+OH radical; 4-7, MeOH extract @ 25, 50, 100 and 200 µg/mL; 8-11, MeOH +DCM extracts @ 25, 50, 100 and 200 µg/mL; and 12, Reactive oxygen species (ROS: OH radical)]

changed as the wavelength changed. The compounds used as standards are shown in Table 3. Gallic acid has several biological functions, including anti-inflammatory, anti-cancer, neuroprotective, anti-fungal, antibacterial and antioxidant properties[41]. In this study, we found that *M. longifolia*, with its gallic acid content (0.64 ppm), could be potential source of gallic acid. Further, we observedhad 48.22 ppm of catechin that has several beneficial biological effects, including antioxidant, antibacterial, antifungal and antialergic properties[42]. Similarly, *M. longifolia* has 98.17 ppm of cinnamic acid known to be antioxidant, antibacterial, anticancer and hepatoprotective[43]; 7.3 ppm of chlorogenic acid which is used to treat ulcers, cancer, oxidative stress and other infections[44]; 10.01 ppm of coumaric acid, known for its antioxidant, antidiabetic, antihyperlipidemic and antibacterial properties[45]; benzoic acid (57.05 ppm) which has antimicrobial, anticancer and antioxidant properties[46]; and 26.27 ppm of hydroxybenzoic acid that shows antioxidant, anticancer, painkilling and inflammation-fighting properties[47]. With such rich content of phenolic compounds, *M. longifolia* is a potential natural source for new drugs with wide applications.

Table 3 — Phenolic compounds (ppm) of *Mentha longifolia*

Phenolic compound	*Mentha longifolia* (ppm)	Retention time
Gallic acid	0.64	17.94281
Chlorogenic acid	7.3	10.65002
Catechin	48.22	62.97033
Hydroxybenzoic acid	26.27	116.20892
Coumaric acid	10.01	151.78816
Benzoic acid	57.05	175.54912
Cinnamic acid	98.17	1075.75012

Table 4 — Chemical composition of *Mentha longifolia*

Compounds	Retention time	Retention index
1-Isopropyl-4-methyl-1,4-cyclohexadiene ($C_{10}H_{16}$)	5.313	2.70
Pulegone ($C_{10}H_{16}O$)	6.678	66.19
Phenol, 2-methyl-5-(1-methylethyl) ($C_{10}H_{14}O$)	7.134	9.22
Caryophyllene ($C_{15}H_{24}$)	8.657	21.89

Further, through GS-MS analysis, we identified four chemicals from the *M. longifolia* extract. The following compounds viz. 1-isopropyl-4-methyl-1,4-cyclohexadiene, pulegone, phenol,2-methyl-5-(1-

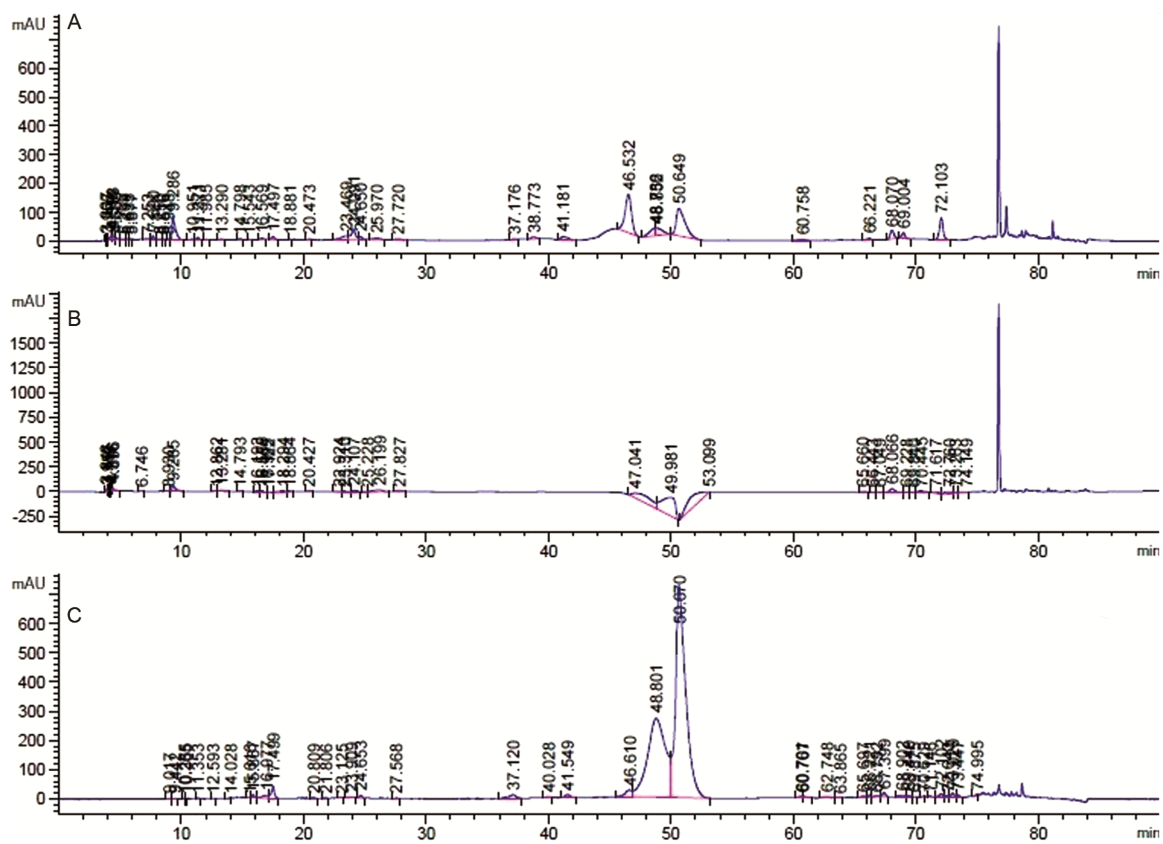

Fig. 3 — HPLC chromatogram of *Mentha longifolia* [A-C: at the wavelength of 278.4; B: 254.4; and 330 nm, respectively; *Peak values on the chromatogram are the peak values of the phenolic compounds determined within the plant. Phenolic compounds used as standards were identified]

methylethyl), and caryophyllene were identified. Caryophyllene has been reported to have anticancer, antimicrobial and antioxidant activities[48], whereas pulegone has antioxidant and antimicrobial potential[49]. The biological activities of the *M. longifolia* extract could be attributed to the compounds detected therein. *Mentha longifolia*, with its rich contents and biological activities, can be a potential natural source for treatment of various ailments.

Conclusion

In our above study, we screened the wild mint, *Mentha longifolia* for phenolic as well as essential oil content, and also determined the biological activities. Our results demonstrated the antioxidant, anti-microbial, antiproliferative and DNA protective activities of the methanolic and dichloromethane extract of its aerial parts. We conclude that *M. longifolia* can be an important source of antioxidants. We observed that the phenolic contents of *M. longifolia* varied between the two different extracts. Overall, *M. longifolia* can serve as a natural resource for pharmacological applications, precisely in drug discovery.

Conflict of interest

Authors declares no competing interests

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