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Original article

The anti-oxidative, anti-cell proliferative and anti-microbial efficacies of cold-adapted *Crepis flexuosa*: HPTLC and GC/MS analyses

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ABSTRACT

The genus *Crepis* constitutes cold-adapted plant spp., of these some are traditionally used in folk medicine against inflammation or fungal infections without scientific validations. Here, we report the biological activities of *Crepis flexuosa* total ethanol-extract (CF-EtOH) and its hexane (CF-Hex), ethyl acetate (CF-EtOA), butanol (CF-ButOH), and aqueous (CF-Aqua) fractions. Our *in vitro* DPPH and ABTS radical-scavenging assays showed CF-EtOH, CF-ButOH and CF-Aqua with maximal, CF-EtOA with moderate, and CF-Hex with mild anti-oxidant activities. When tested on human cancer cell lines, high cytotoxicity was demonstrated by CF-EtOH (IC_{50}: 42.45 μg/ml) and CF-Aqua (IC_{50}: 46.37 μg/ml) on HepG2, followed by CF-Hex (IC_{50}: 63.24 μg/ml) and CF-ButOH (IC_{50}: 65.32 μg/ml) on MCF7 cells. The human primary cell line (HUVEC) had comparatively lower cytotoxicity for the tested samples. Moreover, when assessed for anti-microbial efficacy, CF-ButOH and CF-Aqua exhibited the strongest activity (MIC: 156.25 μg/ml) against *S. aureus*, *E. faecalis* and *C. albicans*. Further, while the developed RP-HPTLC identified the bioactive flavonoid luteolin-7-*O*-glucoside (17.58 mg/g), GS/MS analysis revealed sixteen compounds in *C. flexuosa* extract. In conclusion, we for the first time show the promising anti-oxidative, anti-cell proliferative and anti-microbial efficacies of *C. flexuosa*. This warrants further phytochemical and bio-efficacy studies towards isolations and identifications of active principles.

1. Introduction

Crepis (family: Asteraceae), a large genus of about 200 species of cold-adapted plants is distributed in the northern hemisphere (Mabberley, 2008). Though most of the species are edible, information on their worldwide usage in traditional medicinal is very limited. In Spanish tradition, *C. vesicaria* (Arnica) is used for gastric and arterial circulation problems as well as externally applied for wound healing, bruises and inflammations (Gonzalez-Tejero et al., 1995). In African folk medicine, while *C. carbonaria* is used for increasing the myometrial contractions (Schröder, 1980), *C. cameroonica* (syn. *C. newii*) is used to treat diarrhea, wounds and fungal infections (Ndom et al., 2006). Also, *C. sancta* is consumed as laxative and diuretic supplement in Italy (Sansanelli and Tassoni, 2014).

Crepis flexuosa (Ledeb.) Benth. (syn. *Crepis glouca*, *Barkhausia flexuosa*, *Youngia flexuosa*, *Youngia glouca*) is a shrub with heavily and nearly leafless, forked branches that terminate in a rounded tuft of very small yellow flower heads. It is distributed in Himalayan ranges (3000–4200 m, a.s.l.) of Central Asia, Tibet, central Nepal, western Pakistan and northern India (Ladakh, Himachal Pradesh, Uttrakhand). In the Himalayan Spiti valley of India, *C. flexuosa* juice is used to cure jaundice (Singh and Lal, 2008). However, to our best knowledge, the biological activity of *C. flexuosa* is hitherto not reported. With this background, we for the first time assessed the *in vitro* therapeutic potential of *C. flexuosa*.

2. Materials and methods

2.1. Plant material collection and identification

The whole flowering plant of *C. flexuosa* Benth, locally known as Sili was collected from Shyok-Agham area located between Nubra

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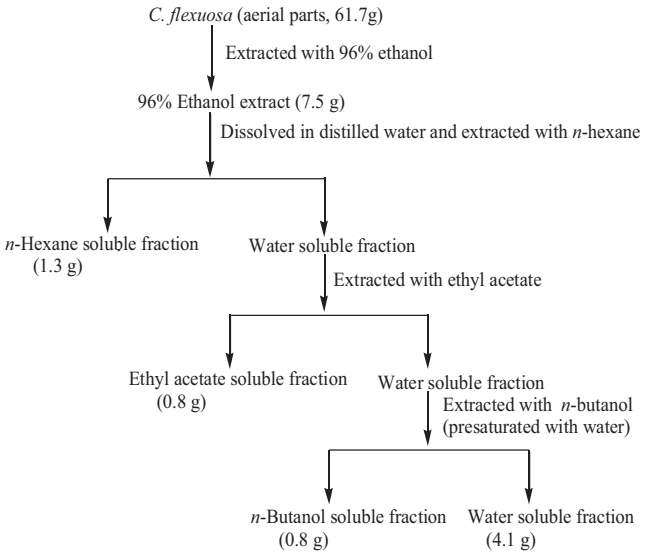
Valley and Pangong (3200 m a.s.l.; Ladakh, India) in July 2018 (Fig. 1). The plant was identified by a local herbalist-Amchi practitioner (voucher specimen no.802018) and further confirmed by Dr. Tariq Husain, a plant taxonomist at National Botanical Research Institute, Lucknow, India.

2.2. Extraction and fractionation

All analytical grade solvents used for extraction and fractionation of *C. flexuosa* were purchased from Sigma Aldrich (Germany). Of these, *n*-hecxane (Hex) and ethyl acetate (EtOAc) were distilled prior to use whereas 96% ethanol (EtOH) and *n*-butanol (BuOH) were used as supplied. The details of procedure adopted for extraction and fractionation is shown (Scheme 1).

Briefly, the dried and grounded (61.7 g) aerial of parts of *C. flexuosa* was extracted with 96% EtOH at RT (3x 200 ml), and filtered (Whatman filter paper no. 1). The solvent was evaporated to dryness at 40 °C *in vacuo* (Buchi Rotavapor; Model R-215) that yielded a syrupy material of EtOH extract (CF-EtOH; 8.1 g). A portion of CF-EtOH (7.5 g) was subjected to liquid–liquid partition successively with Hex, EtOAc and water-saturated BuOH to obtain Hex-soluble fraction (CF-Hex; 1.3 g), EtOAc-soluble fraction (CF-EtOAc; 0.8 g) and BuOH-soluble fraction (CF-ButOH; 0.8 g), including the remaining water-soluble fraction (CF-Aqua; 4.1 g). Solvents were evaporated to dryness as mentioned above for each sample, and kept at 4 °C until analyzed.



Scheme 1.

2.3. Determination of in vitro anti-oxidative activity of C. flexuosa

2.3.1. DPPH radical-scavenging activity

The anti-oxidative activities of CF extract (CF-EtOH) and fractions (CF-Hex, CF-EtOA, CF-ButOH and CF-Aqua) were estimated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method as described elsewhere (Brand-Williams et al., 1995; Hussein et al., 2019). Briefly, 0.5 ml of each test samples (10, 50, 100, 500 and 1000 μg/ml) was mixed with 0.125 ml DPPH and 0.375 ml methanol, and incubated for 0.5 h. Ascorbic acid was used as a positive control and methanol acted as blank. All samples were tested in triplicate and repeated. The optical density (OD; λmax = 517 nm) was recorded using UV–vis spectrophotometer, and the tested samples' free radical-scavenging activies were calculated:

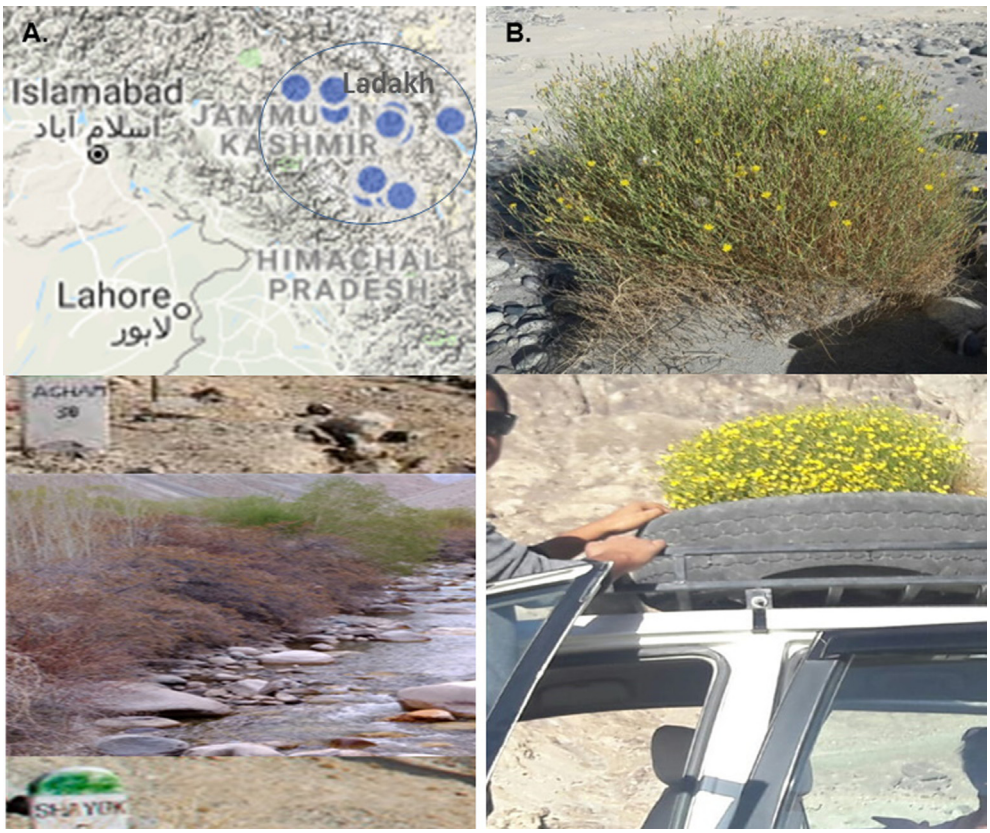


Fig. 1. *Crepis flexuosa* or Sili. (A) Its geographical distribution in high altitude Himalayan ranges (https://indiabiodiversity.org/species/show/259968), and Collection location in Ladakh, India. (B) The whole flowering plant of *C. flexuosa*.

%Radical-scavenging activity = [ODcontrol – (ODsample/ODcontrol)] × 100.

2.3.2. ABTS cation-scavenging activity

The anti-oxidative activities of CF extract (CF-EtOH) and fractions (CF-Hex, CF-EtOA, CF-ButOH and CF-Aqua) were estimated using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method as described elsewhere (Li et al., 2011) with minor modification. Briefly, aqueous solutions of ABTS (7.0 mM) and potassium persulfate (2.45 mM) were prepared separately, and kept in dark for 12 h. The two solutions were mixed and incubated at RT for 30 min, following refrigeration for 24 h, and further dilutions were made in EtOH. To the solution (50 μg/ml; 1:1), different concentration of each sample (10, 50, 100, 500 and 1000 μg/ml) were pipetted to initiate the reaction until a calibration curve was achieved. While ascorbic acid was used as standard, ABTS solution (50 μg/ml) and EtOH (96%) served as negative and control, respectively. All samples were tested in triplicate and repeated. The OD (λmax = 734 nm) was recorded using UV–vis spectrophotometer, and the anti-oxidant activity was determined:

%Radical-scavenging activity = [ODcontrol – (ODsample/ODcontrol)] × 100.

2.4. Assessment of C. flexuosa fractions on cell viability and proliferation

2.4.1. Human cell culture

The three human cancer cell lines MCF7 (breast), HeLa (cervical) and HepG2 (liver) as well a primary umbilical vein endothelial cell (HUVEC-16549) were procured (AITCC, USA) and maintained in DMEM culture media, supplemented with bovine serum (10%) and 1x penicillin–streptomycin mix (all from Invitrogen, USA) at 37 °C with 5% CO_2 supply. The cells ($0.5x10^5$/100 μl/well) were seeded in 96-well flat-bottom cell culture plates (Becton-Dickinson Labware), and grown overnight.

2.4.2. Cytotoxicity assay

The *C. flexuosa* extract (CF-EtOH) and fractions (CF-Hex, CF-EtOA, CF-ButOH and CF-Aqua; 5 mg/ml each) were first dissolved in 100 μg/ml of dimethyl sulfoxide (DMSO; Sigma, Germany) and further diluted in DMEM to make four working concentrations (200, 100, 50 and 25 μg/ml). Overnight grown cells in 96-well plates were treated with each of four doses, including an untreated control (0.1% DMSO) and incubated for 72 h. The anticancer drug dasatanib was used as positive control. The cytotoxicity was evaluated using MTT assay (TACS MTT Cell Proliferation and Viability Assay Kit, USA) according to manufacturer's guidelines, and the absorbance was recorded (Microplate Reader ELx800; BioTek, USA). All samples were tested in triplicate and repeated. Values of 50% maximal cytotoxicity concentration (CC_{50}) were estimated using the best fit regression curve method in Excel (Microsoft, USA).

2.5. Determination of anti-microbial activity of C. flexuosa

2.5.1. Test microorganisms

Two gram-positive (*Staphylococcus aureus*; ATCC 25923 and *Enterococcus faecalis*; ATCC 29212) and two gram-negative (*Escherichia coli*; ATCC 25922 and *Proteus vulgaris*; ATCC 8427) bacterial strains as well as one fungal strain (*Candida albicans*; ATCC 60193) were used in this investigation.

2.5.2. Determination of minimum inhibitory concentrations (MIC)

The MIC for CF extract (CF-EtOH) and fractions (CF-Hex, CF-EtOA, CF-ButOH and CF-Aqua) were assessed using micro-well dilution method as described elsewhere (Mann and Markham, 1998; Sulaiman, 2013) with some modifications. Briefly, duplicated two-fold serial dilutions (2000 through 31.2 mg/ml in DMSO; 5%, v/v) were made using broth media (100 μl/well, final) in 96-well plates, and the microbial suspensions (100 μl; $1x10^6$ CFU/ml) were added. Gentamycin and nystatin were used as positive controls. The plates were incubated for 24 h at 37 °C for bacterial strains, whereas for 72 h at 25 °C fungal strain, respectively. The MIC for each sample was defined as the lowest concentration displaying non-detectable bacterial or fungal growth. For MIC (MBC for bacteria or MFC for fungus) estimation, 5 μl from the wells showing no microbial growth was spread on agar plates and incubated for 24 or 72 h. Values of MBC and MFC therefore, represented the lowest concentrations of tested samples which did not show any sign of bacterial or fungal growth.

2.6. Development of HPTLC method to analyze luteolin-7-O-glucoside in C. flexuosa

The quantification of luteolin-7-*O*-glucoside in *C. flexuosa* extract (CF-EtOH) was carried out on reverse-phase high performance thin layer chromatography (RP-HPTLC; 10 × 10 cm) plate (Merck, Germany). A stock solution of luteolin-7-*O*-glucoside (Sigma, Germany; 1 mg/ml) was prepared in methanol and further diluted to furnish seven different concentrations (20–140 μg/ml). All samples, including *C. flexuosa* extract (6 μl) were gently applied on the TLC plate through a micro liter syringe connected with programmed TLC Sampler-4 (CAMAG, Switzerland) with a band size of 6 mm and at a speed of 160 nl/sec to give linearity range between 200 and 1400 ng/band. The plate was developed in a pre-saturated twin-trough glass chamber (Automatic Development Chamber-2, CAMAG; Switzerland) at specific temperature (25 ± 2 °C) and humidity (60 ± 5%). The developed plate furnished clear and compact spots of luteolin-7-*O*-glucoside as well as different phytoconstituents of *C. flexuosa*, which were quantitatively analyzed at λmax = 254 nm in the absorbance mode.

2.7. GC/MS analysis of C. flexuosa

The chemical constituents of *C. flexuosa* extract (CF-EtOH) were determined utilizing gas chromatography (GC) and a mass spectrometer (MS) (Turbomass, PerkinElmer). The temperature program was adjusted to 40 °C for a 2 min hold, followed by increasing to 200 °C (5 °C/min) and held for 2 min. The temperature was further raised to 300 °C (5 °C/min) and held for another 2 min. The phytochemical composition of CFE was determined by comparing the obtained mass spectra with those of the National Institute of Standard and Technology Spectral Library, the Adams Library (Adams, 2007) and the Wiley GC–MS Library (McLafferty and Stauffer, 1989).

2.8. Statistical analysis

All triplicated data were presented as mean ± standard error, analyzed using one-way analysis of variance and differences between two groups were compared using Student's *t*-test (SPSS software; Version 25; IBM, USA). $p < 0.05$ was considered significant.

3. Results

3.1. Anti-oxidative activities of C. flexuosa extract and fractions

Results of both DPPH and ABTS assays showed dose-dependent scavenging activities by *C. flexuosa* extract and fractions (Table 1). Of the tested samples, CF-EtOH (ethanol-extract), CF-ButOH

Table 1
Radical-scavenging activities of *Crepis flexuosa* extract and fractions.

Samples	Concentrations (μg/ml)				
	10	50	100	500	1000
DPPH radical-scavenging activity (%)					
CF-EtOH	11.6 ± 0.9	19.2 ± 1.2	33.2 ± 1.8	52.1 ± 2.2	67.2 ± 2.1
CF-Hex	2.4 ± 0.3	9.2 ± 3.1	18.6 ± 0.8	30.6 ± 2.2	43.6 ± 1.2
CF-EtOA	7.3 ± 2.8	12.2 ± 0.9	25.1 ± 2.2	39.2 ± 1.4	50.6 ± 2.3
CF-ButOH	15.6 ± 1.4	28.8 ± 2.8	38.8 ± 2.4	51.3 ± 2.9	69.6 ± 2.6
CF-Aqua	17.5 ± 0.4	31.3 ± 0.3	41.1 ± 1.2	54.3 ± 0.2	70.2 ± 1.9
Ascorbic acid	80.7 ± 2.0	85.1 ± 1.3	85 ± 1.2	88.7 ± 2.4	90.7 ± 1.4
ABTS cation-scavenging activity (%)					
CF-EtOH	10.2 ± 0.7	17.1 ± 1.2	23.3 ± 1.1	45.1 ± 1.5	64.1 ± 1.2
CF-Hex	2.3 ± 1.9	5.2 ± 2.1	10.7 ± 1.9	21.8 ± 2.1	39.3 ± 1.7
CF-EtOA	5.8 ± 2.3	10.2 ± 1.9	15.1 ± 1.2	26.9 ± 1.6	47.9 ± 2.6
CF-ButOH	10.1 ± 0.9	18.3 ± 2.9	25.9 ± 1.9	47.1 ± 2.4	68.3 ± 2.3
CF-Aqua	11.5 ± 0.6	20.4 ± 0.9	28.1 ± 2.2	51.1 ± 0.9	69.1 ± 1.5
Ascorbic acid	80.7 ± 2.4	81.2 ± 2.1	84.2 ± 1.9	87.2 ± 2.4	88.7 ± 2.1

CF: *Crepis flexuosa*; EtOH; ethanol-extract; Hex: hexane-fraction; EtOA: ethyl acetate-fraction; ButOH: butanol-fraction; Aqua: aqueous-fraction. Means of three determinants (±SD).

(butanol-fraction) and CF-Aqua (aqueous-fraction) exhibited the highest anti-oxidant activities in both assays (Table 1). Comparatively, CF-EtOA (ethyl acetate-fraction) and CF-Hex (hexane-fraction) showed moderate and mild anti-oxidant activity, respectively.

3.2. Effects of C. flexuosa extract and fractions on cancer cell viability

The *C. flexuosa* extract and fractions when tested on a panel of human cell lines, showed differential cytotoxicity even at the lowest dose (25 μg/ml). Of these, the highest cytotoxicity was demonstrated by the ethanol-extract (IC_{50}: 42.45 μg/ml) and aqueous-fraction (IC_{50}: 46.37 μg/ml) on liver cancer cells followed by hexane-fraction (IC_{50}: 63.24 μg/ml) and butanol-fraction (IC_{50}: 65.32 μg/ml) on breast cancer cells (Table 2). However, though the ethyl acetate-fraction did not show any cytotoxicity at 100 μg/ml, it exhibited high toxicity at 200 μg/ml (Table 2). The HUVEC cells demonstrated comparatively lower cytotoxicity for all tested samples. Notably, while the observed non-cytotoxicity by the ethyl-acetate fraction coincided with its anti-oxidative activity, the high toxicity of hexane-fraction corresponded to its poor anti-oxidant activity.

3.3. Anti-microbial efficacies of C. flexuosa extract and fractions

The *C. flexuosa* extract and fractions showed variable degrees of inhibitory effects on both bacterial and the fungal strains with MIC values in the range of 156.25–1250.0 (Table 3). Of the tested samples, the butanol-extract and aqueous-extract had the best activities against *S. aureus* and *E. faecalis* (MIC: 156.25 μg/ml) compared to others. On the other hand, the ethyl acetate-fraction and hexane-fraction showed weak activities against *S. aureus* and *E. faecalis* and no activity against *E. coli* and *P. vulgaris*. Both the butanol-fraction and aqueous-fraction showed strong activities (MFC: 156.5 μg/ml) against *C. albicans* as compared to others (Table 3).

Table 2
The estimated CC_{50} (μg/ml) values of *Crepis flexuosa* extract and fractions on cultured human cancer cell lines.

Samples	MCF7	HeLa	HepG2	HUVEC
CF-EtOH	56.18	54.27	42.45	76.34
CF-Hex	63.24	74.42	68.13	82.62
CF-EtOA	216.14	224.62	208.62	267.36
CF-ButOH	65.32	75.25	75.42	92.23
CF-Aqua	68.27	66.17	46.37	81.73
DAS	6.35	7.39	4.66	18.75

CF: *Crepis flexuosa*; EtOH; ethanol-extract; Hex: hexane-fraction; EtOA: ethyl acetate-fraction; ButOH: butanol-fraction; Aqua: aqueous-fraction. DAS: Dasatanib; Means of three determinants (±SD).

3.4. Quantitative HPTLC analysis of luteolin-7-O-glucoside in C. flexuosa extract

Of the various combinations of mobile-phase solvents tested for the quantitative RP-HPTLC analysis of luteolin-7-*O*-glucoside in *C. flexuosa* extract, a combination of acetonitrile:water (40:60; v/v) was found as the most suitable mobile-phase. The pictograms of the developed TLC plate (Fig. 2A) and 3D of all tracks (Fig. 2B) are presented. The developed method furnished intense peaks of luteolin-7-*O*-glucoside (peak-1; R_f = 0.59) (Fig. 2C) while clearly separating the standard (peak-1) and luteolin-7-*O*-glucoside

Table 3
The estimated minimal inhibitory concentrations (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) of *Crepis flexuosa* extract and fractions.

Samples	Activity	*S. aureus*	*E. faecalis*	*E. coli*	*P. vulgaris*	*C. albicans*
CF-EtOH	MIC	625	625	–	–	156.25
	MBC	1250	1250	–	–	NT
	MFC	NT	NT	NT	NT	312.5
CF-Hex	MIC	625	625	–	–	156.25
	MBC	1250	1250	–	–	NT
	MFC	NT	NT	NT	NT	312.5
CF-EtOA	MIC	625	312.5	625	625	156.25
	MBC	1250	625	1250	1250	NT
	MFC	NT	NT	NT	NT	312.5
CF-ButOH	MIC	156.25	156.25	625	312.5	78.12
	MBC	312.5	312.5	1250	625	NT
	MFC	NT	NT	NT	NT	156.25
CF-Aqua	MIC	156.25	156.25	625	312.5	78.12
	MBC	312.5	312.5	1250	625	NT
	MFC	NT	NT	NT	NT	156.25
Gentamycin	MIC	7.8	7.8	3.9	3.9	NT
	MBC	15.6	15.6	7.8	7.8	NT
Nystatin	MIC	NT	NT	NT	NT	3.5
	MFC	NT	NT	NT	NT	7.0

CF: *Crepis flexuosa*; EtOH; ethanol-extract; Hex: hexane-fraction; EtOA: ethyl acetate-fraction; ButOH: butanol-fraction; Aqua: aqueous-fraction. Not tested (NT); No activity (-).

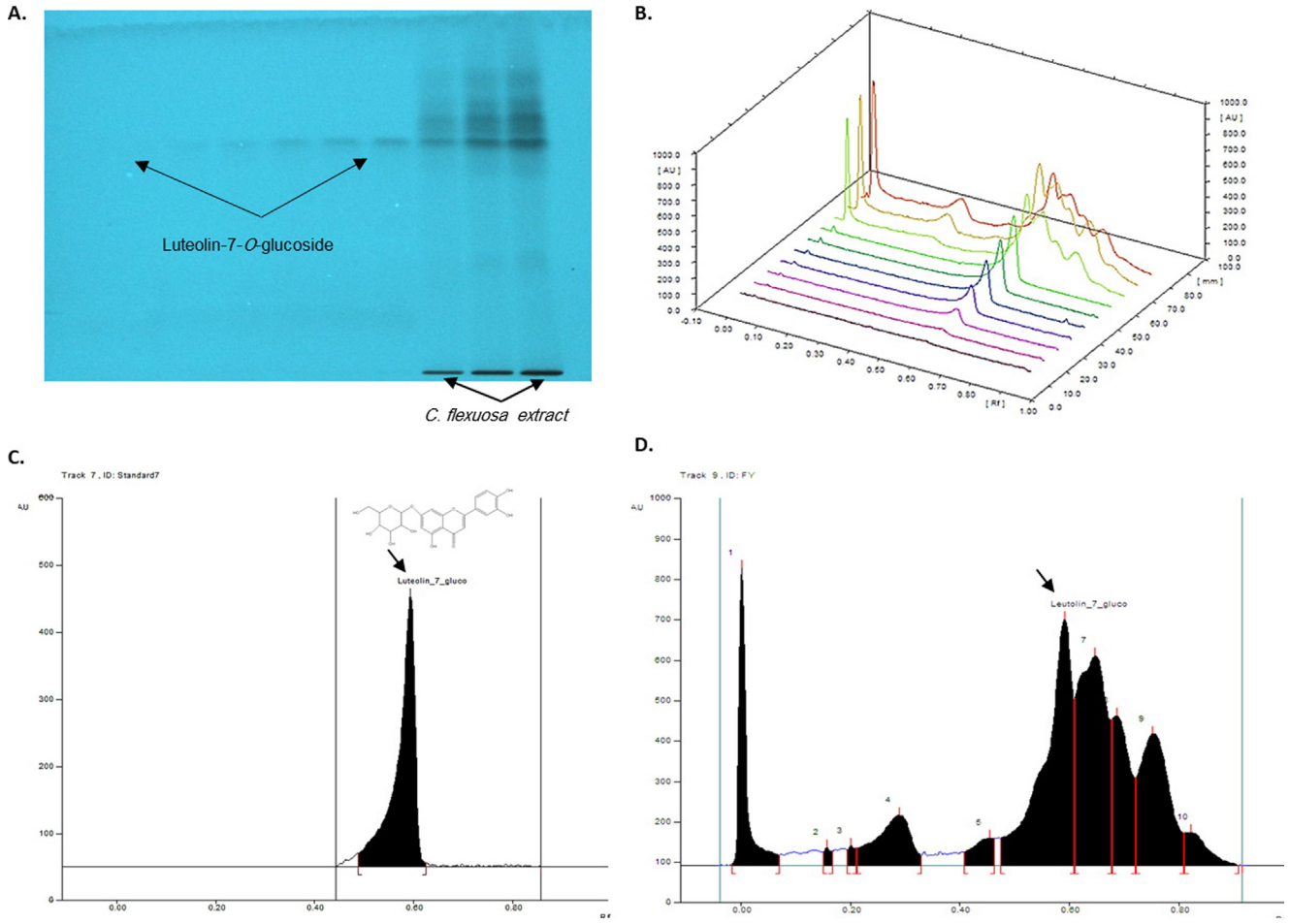


Fig. 2. Quantification of luteolin-7-*O*-glucoside in *Crepis flexuosa* ethanol-extract by validated RP HPTLC (λ = 254 nm; mobile phase- acetonitrile:water, 40:60, v/v). (A) Pictogram of developed HPTLC plate. (B) 3 D display of all tracks. (C) Chromatogram of standard luteolin-7-*O*-glucoside (peak-1). (D) Chromatogram of *C. flexuosa* containing luteolin-7-*O*-glucoside (peak-6).

(peak-6), including different phytoconstituents in the *C. flexuosa* extract (Fig. 2B). The ratio of regression equation (*y*) and correlation co-efficient (r^2) for luteolin-7-*O*-glucoside was found to be y = 4.8846x − 755.18/0.988. The developed HPTLC method led to the quantification of luteolin-7-*O*-glucoside (17.58 mg/g) in the dried weight of *C. flexuosa* extract.

3.5. Phytochemical composition of C. flexuosa

Sixteen compounds were identified in *C. flexuosa* by GC–MS with their retention time, chemical formula, molecular weight, and concentration (Table 4; Fig. 3). The identified compounds in *C. flexuosa* were represented based on their elution order on the

Table 4
GC/MS profiling of identified compounds in *Crepis flexuosa* ethanol-extract.

Compound Name	*Formula*	*MW (g/mol)*	*RT* (min)	*Area (%)*
N-Methoxy-N-methylacetamide	$C_4H_9NO_2$	103.12	3.32	27.610
Isoamyl alcohol	$C_5H_{12}O$	88.15	3.60	2.410
Glycerol	$C_3H_8O_3$	92.09	3.70	6.860
Methyl acetate	$C_3H_6O_2$	74.08	3.83	4.090
DL-Glyceraldehyde dimer	$C_6H_{12}O_6$	180.16	5.67	4.790
3,7,7-Trimethyl-1,3,5-cycloheptatriene	$C_{10}H_{14}$	134.22	8.79	0.700
1,2,3-Propanetriol monoacetate	$C_5H_{10}O_4$	134.13	11.08	5.120
ihydro- 2,3-Dihydro-3,5- dihydroxy-6-methyl-4H-pyran-one	$C_6H_8O_4$	144.12	11.61	6.860
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	12.11	1.560
4-Hydroxymethylbenzaldehyde	$C_8H_8O_2$	136.15	14.07	1.850
4-Hydroxyphenylacetic acid	$C_8H_8O_3$	152.15	15.66	11.930
2-Decen-1-ol	$C_{10}H_{20}O$	156.26	19.16	0.990
Palmitic acid	$C_{16}H_{32}O_2$	256.42	20.44	3.640
Undecane	$C_{11}H_{24}$	156.31	25.27	1.370
Nonacosanol	$C_{29}H_{60}O$	424.8	28.13	3.500
Octadecane	$C_{18}H_{38}$	254.5	31.47	0.810

RRI, relative retention indices calculated against n-alkanes; %, calculated from the flame ionization detector (FID) chromatograms; tr, trace (<0.1%). Identification method: tR, identification based on the retention times (tR) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and compared with literature data.

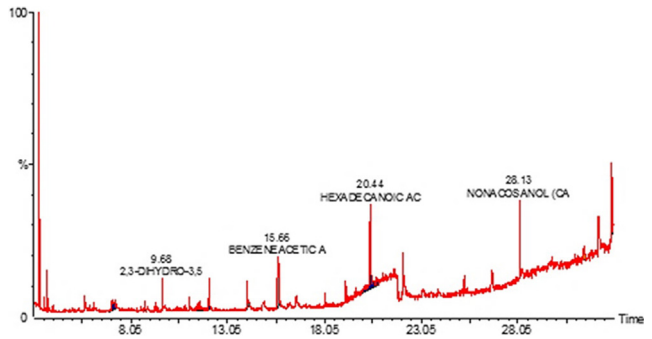


Fig. 3. GC/MS chromatogram of *Crepis flexuosa* ethanol-extract.

HP Innowax column. Of these, N-methoxy-N-methylacetamide (27.6%) and 4-hydroxyphenylacetic acid (11.9%) were the primary and moderate constituents, respectively. The other identified compounds were present in fairly good amounts.

4. Discussion

The genus *Crepis* constitutes species of cold-adapted high altitude plants (Mabberley, 2008), of which some are traditionally used for jaundice, wound healing, inflammations or fungal infections (Gonzalez-Tejero et al., 1995; Schröder, 1980; Ndom et al., 2006; Sansanelli and Tassoni, 2014; Abbas et al., 2017). Nonetheless, information on their medicinal usage remains very limited and without scientific rationale or experimental validations. In the Himalayan valley of Ladakh and neighboring regions, *C. flexuosa* juice is used to cure jaundice (Singh and Lal, 2006). However, to the best of our knowledge, the bio-efficacy of *C. flexuosa* is hitherto not reported. In this report, we for the first time evaluated the *in vitro* biological activities of *C. flexuosa* ethanol-extract as well as its hexane, ethyl acetate, butanol and aqueous fractions.

Accumulations of indigenous reactive-oxygen species (ROS) damage lipids, proteins or nucleic acids leading to promote oxidative stress and tissue damages (Opara, 2006; di Bello et al., 2018). Our *in vitro* DPPH and ABTS radical-scavenging assays showed *C. flexuosa* ethanol-extract, butanol-fraction and aqueous-fraction with the highest, whereas ethyl acetate-fraction with moderate and hexane-fraction with mild anti-oxidant activities. However, when tested on human cancer cell lines, ethanol-extract (IC_{50}: 42.45 µg/ml) and aqueous-fraction (IC_{50}: 46.37 µg/ml) demonstrated highly cytotoxic effect on liver cancer cells, followed by that of hexane-fraction (IC_{50}: 63.24 µg/ml) and butanol-fraction (IC_{50}: 65.32 µg/ml) on breast cancer cells. However, compared to the cancer cells, they had lower cytotoxic effects on primary umbilical endothelial cells.

Subsequently, *C. flexuosa* extract and fractions when tested for their anti-microbial activities against bacterial (*S. aureus*, *E. faecalis*, *E. coli* and *p. vulgaris*), and fungal (*C. albicans*) strains showed differential activities. Notably, the butanol and aqueous extracts exhibited the highest efficacy (MIC: 156.25 µg/ml) against *S. aureus*, *E. faecalis* and *C. albicans*. This strongly endorses the traditional use of *Crepis* spp. in fungal infections (Ndom et al., 2006).

Luteolin and its derivatives have been identified as the most commonly found flavonoids with a wide range of therapeutic potentials (Sareedenchai and Zidorn, 2010). Of these, their apoptotic, anti-carcinogenic, anti-tumorigenic or cytotoxic as well as anti-oxidative activities via ROS inhibition have been documented (Lopez-Lazaro, 2009). In previous phytochemical studies, luteolin derivatives including quercetin have been reported in both *C. divaricata* and *C. pygmaea* (Rees S and Harborne, 1984). Moreover, leuteolin and luteolin-7-*O*-glucoside have been demonstrated for their anti-oxidative as well as anti-inflammatory salutations (Seelinger at al., 2008; Park and Song, 2013). In line with this, we also report HPTLC based quantification of luteolin-7-*O*-glucoside in *C. flexuosa*. In addition, our GC/MS analysis has identified sixteen compounds in *C. flexuosa*. Of these, 3,7,7-trimethyl-1,3,5-cycloheptatriene has been reported in cold-adapted Scots pine and pine beetles (Gries et al., 1992).

5. Conclusion

Our data for the first time, demonstrated the promising anti-oxidative, anti-cell proliferative and anti-microbial therapeutic potential of *C. flexuosa*, including identification of bioactive luteolin-7-*O*-glucoside in its extract. This warrants further phytochemical and bio-efficacy studies towards isolations and identifications of active principles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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