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Original article

Phytochemical profiling, antioxidant and antibacterial efficacy of a native Himalayan Fern: *Woodwardia unigemmata* (Makino) Nakai

Poonam Takuli [a], Kapil Khulbe [a,*], Parikshit Kumar [a], Archana Parki [b], Asad Syed [c], Abdallah M. Elgorban [c]

[a] Department of Botany, D.S.B. Campus, Kumaun University, Nainital 263001, Uttarakhand, India
[b] Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar U.S. Nagar 263145, Uttarakhand, India
[c] Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

Present work elucidates the antioxidant and antibacterial activity of *Woodwardia unigemmata* (Makino) Nakai along with chemical characterization using its aqueous (AEW), methanol (MEW), and hexane (HEW) extracts. Chemical profile of different extracts was illustrated by using Gas chromatography and mass spectrometry (GC-MS) analysis. Antioxidant activities were tested using DPPH and FRAP assays, total phenolic and flavonoid content by Folin-Ciocalteu and aluminum chloride method, respectively. Further, antibacterial activity against six plant and four animal pathogenic bacteria was analyzed by employing the disc diffusion assay. GC-MS analysis revealed the presence of catechol (21.96%), glycerol (20.22%), n-pentadecanoic acid (6.95%), glyceryl monoacetate (6.35 %), ethyl acetimidate (5.39 %) and 3-hydroxy-2,3-dihydromaltol (5.36%) in AEW; β-sitosterol (17.39%), pentadecanoic acid (9.81%), vitamin E (7.82%) and glycerol (7.05%) in MEW; γ-sitosterol (33.45%), vitamin E (10.04%) and campesterol (7.32%) in HEW as major constituents. Maximum phenolics (873 ± 6.01 mgGAE/g dry extract) as well as flavonoids (151 ± 11.44 mgQE/g dry extract) content was found in MEW, which also showed remarkable antioxidant potential (IC_{50} 6.07 ± 1.4 μg/ml for DPPH and 768 ± 10.4 mg AAE/g dry extract for FRAP assay. In antibacterial activity, maximum inhibition (15 ± 0.9 mm) was observed for HEW against *R. solanacearum*, followed by AEW against *A. tumefaciens* and *X. phaseoli* (11 ± 0.3 mm each). MEW was found positive only against *A. tumefaciens*. Significant minimum inhibitory concentration (MIC) value observed for AEW against *L. monocytogenes* (10 mg/ml). Polar extracts had remarkable antioxidant potential, while non-polar extract did show significant antibacterial activity. Further, GC- MS reports indicated that this traditionally useful fern species can be an excellent source of biologically active compounds.

1. Introduction

Woodwardia unigemmata (Makino) Nakai, commonly known as jewelled chain fern belongs to family Blechnaceae. So far, thirteen (Gasper et al., 2016) to fourteen (Kramer et al., 1990; Cranfill and Kato, 2003) species of the genus *Woodwardia* have been recognized all over the world, out of which *W. unigemmata* is the single species of this genus in India (Fraser-Jenkins et al., 2017). This species is predominantly distributed in the northern hemisphere, especially in eastern Asia (Kramer et al., 1990). It is evergreen fern with arching, bipinnately-divided fronds reaching up to 1.5 m in length. In its native habitat it grows as a forest understory plant. It is terrestrial and lithophytic, grows well in beds of hill ravines. It is widely distributed throughout Himalayan Region usually between 1200 and 2400 m altitude.

There is a vast literature on traditional and economic uses of this plant. The decoction of rhizome and fronds internally administrated in dysentery, dried rhizome used as purgative, fronds used in skin diseases, and infertility (Gaur and Bhatt, 1994; Nwosu, 2002). Starch extracted from rhizomes is used to prepare cake, noodles, and liquor in China (Dai et al., 2003; Yun et al., 2009; Liu et al., 2012). In combination with some other ferns, it is used for the preparation of Chinese herbal preparation (Blechni Rhizoma). This Rhizoma has been traditionally used to treat hepatitis,

* Corresponding author.
E-mail addresses: kapilkhulbebot@kunainital.ac.in (K. Khulbe), assyed@ksu.edu.sa (A. Syed), aelgorban@ksu.edu.sa (A.M. Elgorban).
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Human Immunodeficiency Virus (HIV), injury, swelling, fever, measles, and erysipelas (Tsai and Hwang, 1999).

Previously, various natural biologically active compounds have been discovered from different species of this genus (Gonzalez et al., 2000; Hanus et al., 2003; Hiroshi et al., 1992; Zhong et al., 2008) including *W. unigemmata*. Four stenones including ecdysternone, ponasteroside A, woodwardic acid, and two flavonoids were isolated from the ethanol extract of *W. japonica*, (Luan et al., 2002; Liu and Gao, 2011). Two cyclohexenone glycosides were isolated from American fern *W. virginica* (Rezanka et al., 2003). Recently, new glucoside, woodorien was isolated from *W. orientalis* extract, which was the most potent inhibitor of type I Herpes simplex virus (Xu et al., 1993).

Only a few reports are available on antioxidant activity Zhong et al. (2008) and phytochemical constituents Rui et al. (2017) of *W. unigemmata*. Plant-based chemical compounds have a wide range of industrial and pharmacological uses. These reports indicate that this genus restrains different biologically active compounds. With this view, the present study has been taken up for studying the phytochemical profile, antioxidant and antibacterial activity of different polar and non-polar solvent (aqueous, methanol, and hexane) extracts of jewelled chain fern.

2. Material and methods

2.1. Collection and extraction of plant material

The fresh aerial part of *W. unigemmata* (Makino) Nakai was collected from Central Himalaya, India (1725 m asl; 29.8378° N, 80.1861° E) following Jain and Rao (1977). Taxonomic identification of the specimen (KU-BOT-WU02) was made by plant taxonomists, Late Prof. Y.P.S. Pangtey and Prof. P.C. Pandey, Department of Botany, D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India, and further authenticated by C.R. Fraser Jenkins. Solvent extraction was done following Handa et al. (2008), with some modification: Freshly collected plant material was thoroughly washed and shade dried (25 ± 2 °C) under sterilized conditions. After complete the drying process, the dried parts were powdered using an electric grinder. Powdered samples (25 g) were then immediately soaked in 150 ml each of aqueous, methanol and hexane for 36hr in a shaker (120 rpm). Extracts were then filtered and concentrated to dryness using a rotary evaporator at 35°C, and stored in the dark at 4°C for the further analysis.

2.2. Chemical characterization via GC-MS analysis

Three extracts of *W. unigemmata* (MEW- methanol extract, AEW- aqueous extract, and HEW- hexane extract) were analyzed by GC/MS analysis using GC MS-QP Plus, equipped with an Rtx-5 MS capillary column (0.25 mm film thickness, 0.25 mm internal diameter, and 30 m in length) (J&W Scientific, Agilent, Santa Clara, CA, USA) with helium as carrier gas: with a flow rate of 1.21 ml/min. The initial oven temperature was set at 100 °C for 2 min, and finally to 280 °C with a rate of 10 °C/min. The components were identified by comparing the spectral data of peaks with the corresponding standard mass spectra of NIST-MS and Wiley library.

2.3. Total phenolic content

Total phenolics were estimated by following Folin- Ciocalteu Regent (FCR) method of Singleton and Rossi (1965) with little modification. Different concentrations (10–100 μg/ml) of standard and test samples were prepared to form the stock solution. 0.5 ml of each concentration was mixed with 0.2 ml of FCR, 0.5 ml Na_2CO_3 (20%). After 30 min of incubation at room temperature, absorbance was recorded at 765 nm against the blank. The concentration of total phenolic content was calculated as mg Gallic acid equivalent/g of dry extract, from the calibration curve of the standard solution of Gallic acid ($Y = 0.004x$; $R^2 = 0.995$).

2.4. Total flavonoid content

Total Flavonoids were determined using the $AlCl_3$ method with some modifications (Willet, 2002). Different dilutions (10–100 μg/ml) of Quercetin (standard) and tests were prepared. Each sample (0.5 ml) was mixed with 0.1 ml Aluminium chloride (10%), 1 M Potassium acetate (0.1 ml), raising the final volume to 5 ml by adding distilled water. After incubation at room temperature for 30 min, the absorbance was recorded at 415 nm. Based on the Quercetin calibration curve ($Y = 0.006x$; $R^2 = 0.983$), the flavonoid content was calculated and articulated in terms of mg Quercetin equivalents/ g of dry extract.

2.5. Antioxidant activity

Antioxidant potential of different extracts of *W. unigemmata* (HEW, MEW and AEW) were screened using DPPH (2,2-diphenyl- 1-picryl-hydrazyle) scavenging assay and FRAP (ferric reducing antioxidant power) assay.

2.6. DPPH assay

Scavenging of free radical (DPPH) by different extracts was determined by following Xie et al. (2010) with some modifications. 2 ml of fresh DPPH – methanol solution (0.1 mM) was mixed with 2 ml of the plant extract at different concentrations (10–100 μg/ml). The reaction mixture was incubated in the dark for 30 min at room temperature and analyzed spectrophotometrically at 517 nm against blank. Ascorbic acid was used as the positive control. The free radical scavenging potential of plant extracts was estimated using the formula:

% Inhibition = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] × 100

IC_{50} value of test samples and standard was determined using the percentage inhibition versus concentration graph.

2.7. FRAP assay

Ferric Reducing Antioxidant Power (FRAP) assay was carried out following Benzie and Strain (1999) with slight revision. Extracted samples (100 μl were mixed with FRAP reagent (2.5 ml) in a test tube. Both samples and blank were incubated at 37^0C for 10 min, and absorbance of the sample was recorded against blank at 593 nm. A series of 10 to 100 μl were prepared using an aqueous solution of ascorbic acid as standard. On the basis of ascorbic acid calibration curve ($Y = 0.022x$; $R^2 = 0.992$), the FRAP values were articulated as mg ascorbic acid equivalent / g of dry extract.

2.8. Microorganisms used

Four animal pathogenic (*Klebsiella pneumoniae* MTCC No.7028, *Listeria monocytogenes* MTCC No. 657, *Pseudomonas aeruginosa* MTCC No.3542, *Proteous mirabilis* MTCC No. 3310; from Microbial Type Culture Collection - IMTECH, Chandigarh, India), and six plant pathogenic (*Agrobacterium tumefaciens* MTCC No.609, *Ralstonia solanacearum* ITCC No. BH0007, *Xanthomonas campestris* ITCC No. BD0006, *Xanthomonas oryzae* ITCC No. PI0012; *Xanthomonas phaseoli* and *Erwinia chrysanthemi* from Indian Agriculture Research Institute, New Delhi, India, and, Plant Pathology Department, G. B. Pant University, Pantnagar, India) bacterial strains were used.

Respective stock culture (1% v/v) was inoculated using nutrient agar broth and activated at 37°C for 18 h for further use (Andrews, 2005).

2.9. Screening for antibacterial activity

Disc diffusion method was deployed for antibacterial tests of selected microorganisms (Bauer et al., 1966). Nutrient agar plates (9 cm size) were prepared and cooled down to 20 ± 2 °C. Test bacterial inoculums 100 µl containing 10^6 CFU/ml of test bacteria were spread uniformly by bacterial spreader. Each organism (culture) was inoculated on three (3) plates (replicate). 20 µl of extract (50 mg/ml) was loaded onto the sterile discs (6 mm). These loaded discs were then placed on the surface of the inoculated agar plates at equidistance, and then these plates were incubated at 37 ± 1 °C for 24 h. Positive (Gentamicin −10 mcg, ampicillin −10 mcg) as well as negative controls (respective solvent) were also used. After incubation (24 h) of nutrient agar plates, a clear halo zone around the discs signified a positive antimicrobial activity, and the zone diameter expressed in millimeters including disc size (Vineela and Elizabeth, 2005). The mean of the halo zone diameter (±SD) was accounted for the antibacterial activity of the extracts.

2.10. Evaluation of minimum inhibitory concentrations (MIC's)

The lowest concentration which is responsible for the visible inhibition of the microbial growth is defined as MIC. In present analysis, plant extracts which were found efficient at screening (50 mg/ml) were further analyzed using different dilutions (30, 20, 10, 0.5, and 0.25 mg/ml) following two fold serial dilution technique (Zaidi et al., 2009). The loaded filter paper discs with requisite concentrations were placed on agar plates, which in turn were then incubated at 35 °C (24 h). The halo zones were measured and noted with respect to the effective concentrations.

2.11. Statistical analysis

All experiments were done in triplicates, and results obtained were expressed as mean ± standard error (SE). Analysis of correlation coefficients of determination was calculated by using SPSS (version 16 for window).

3. Results

3.1. GC-MS analysis of different extracts of W. unigemmata

Outcome of the GC-MS results affirm the presence of different bioactive compounds in all three extracts. Different proportions of major constituents (>5%) highlights the influence of solvents on extractability. Investigation of AEW reveal the existence of 26 compounds, representing 88% of the total volume (Table 1) with catechol (21.96%), glycerol (20.22%), n-pentadecanoic acid (6.95%), glyceryl monoacetate (6.35%), ethyl acetimidate (5.39%), and 3-hydroxy-2,3-dihydromaltol (5.36%) as major components. As indicated in Table 2, β- sitosterol (17.39%), pentadecanoic acid (9.81%), vitamin E (7.82%), and glycerol (7.05%) are the major contributors of entire 24 constituents (representing 82% of total volume) reported for MEW. Similarly, HEW displayed a total of 15 compounds, comprising 72.42% of total fraction with γ-sitosterol (33.45%), vitamin E (10.04%), and campesterol (7.32%) as its prominent compounds (Table 3).

3.2. Quantitative phytochemical analysis

Results for total polyphenolic compounds (total phenolic content –TPC; total flavonoid content -TFC) are summarized in Table 4. When comparing the TPC content in terms of mg GAE gm^{-1} dry extract, MEW was found to show a higher TPC value (873 ± 6.01) followed by AEW (363 ± 7.27) and HEW (31.67 ± 10.94). A similar trend was also noted for the TFC (in terms of mg QE gm^{-1} dry extract) among extracts, i.e., MEW (151 ± 11.24) has a higher TFC compared to AEW (146 ± 7.84) and HEW (43.89 ± 1.47).

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

The free radical scavenging activity of different extracts is shown in Table 4 and Fig. 1. MEW exhibited most effective inhibition activity (97.26%) for DPPH radical at 100 µg/ml concentration with $IC_{50,}$ 6.07 ± 1.4 µg/ml. A moderate radical scavenging activity (81.65% at 100 µg/ml; IC_{50}-50.68 ± 2.5 µg/ml) was observed by AEW, followed by HEW (32.98% at 100 µg/ml; IC_{50}, 87.27 ± 7.3 µg/ml).

3.3.2. FRAP assay

The reducing activity (Fe^{+3} to Fe^{+2}) of AEW, MEW, and HEW are presented in Table 4. Here also, MEW showed greater FRAP value as 768 ± 10.4 in terms of mg AAE gm^{-1} dry extract. AEW and HEW demonstrated a significant difference in comparison with MEW, with FRAP value of 605 ± 7.08 and 85 ± 5.13 mg AAE gm^{-1} dry extract, respectively.

3.4. Antibacterial activity of different extracts

In vitro antibacterial activity of *W. unigemmata* extracts were studied against animal and plant pathogenic bacterial strains, as presented in Table 5.

HEW displayed the broad-spectrum antibacterial activity against tested bacterial strains. Its highest inhibitory effect was observed against *R. solanacearum* (ZOI- 15 ± 0.9 mm, MIC-30 mg ml^{-1}), moderate against *X. oryzae* (10 ± 0.7 mm, MIC-30 mg ml^{-1}) and *L. monocytogenes* (10 ± 3.9 mm, MIC 10 mg ml^{-1}) and comparatively less against *X. phaseoli* (9 ± 0.3 mm), *A. tumefaciens* (8 ± 0.0 mm), *E. chrysanthemi* (8 ± 2.6 mm), *X. campestris* (7 mm) and *K. pneumoniae* (7 mm). AEW was found moderately active against five tested bacterial strains (Table 5). Further, all tested bacterial strains except *A. tumefaciens* (ZOI, 8 mm) were found resistant to MEW.

4. Discussion

Analyzing PSMs, together with preliminary clinical traits, have rightfully gained dominant importance in validating their acclaimed health vitalizing effects. The present investigation supports previous findings for ferns having biologically active PSMs (Liu et al., 1986; Mishra and Verma, 2010; Laware and Limaye, 2012; Chowdhary et al., 2010; Francisco and Driver, 1984; Harada and Saiki, 1955; Hu et al., 2008; Li et al., 2014; Ma and Gang, 2004; Ma et al., 2003). Major components observed in this study are well documented in earlier reports. Catechol, individually, as an antioxidant agent, has significant activity against degenerative diseases (Berberian et al., 2007). Also, its antimicrobial properties, as well as contribution in various metabolic processes are well documented (Jeong et al., 2009; Robinson et al., 1992; Sigma–Aldrich, 2006). Glycerol, a most versatile and valuable chemical, also has application as an additive (Bonnardeaux et al.,

Table 1
Chemical composition of aqueous extract of *W. unigemmata* (AEW).

S.No.	Compound	Molecular Formula	Retention Time	Molecular Weight	Nature of Compound	% Contribution
1	methyl 2-ethylacetoacetate	$C_7H_{12}O_3$	4.59	144	Ester	0.99
2	ehtyl acetimidate	C_4H_9NO	4.94	87	Ester	5.39
3	2-hydroxy-2-cyclopenten-1-one	$C_5H_6O_2$	2.34	98	Ketone	2.34
4	Glycerol	$C_3H_8O_3$	7.25	92	Alcohol	20.22
5	acetopropionic acid	$C_5H_8O_3$	8.02	116	fatty acid	1.09
6	propyl acetate	$C_5H_{10}O_2$	8.19	102	Ester	0.70
7	tetraacetic acid lactone	$C_8H_8O_4$	8.83	168	Ketone	1.22
8	α-methylacetoacetic ester	$C_7H_{12}O_3$	9.92	144	Ester	0.83
9	3-hydroxy-2,3-dihydromaltol	$C_6H_8O_4$	10.10	144	Ketone	5.36
10	Catechol	$C_6H_6O_2$	11.30	110	Phenol	21.96
11	glyceryl monoacetate	$C_5H_{10}O_4$	12.14	134	Alcohol	6.35
12	acetopropyl acetate	$C_7H_{12}O_3$	12.94	144	Ester	3.03
13	3-methoxyacetophenone	$C_9H_{10}O_2$	13.23	150	Ketone	0.66
14	pyrogallol	$C_6H_6O_3$	14.92	126	Phenol	1.19
15	3-methylsalicylaldehyde	$C_8H_8O_2$	15.70	136	Aldehyde	1.24
16	3-oxo-α-ionol	$C_{13}H_{20}O_2$	18.49	208	sesquiterpenoid	0.59
17	Loliolide	$C_{11}H_{16}O_3$	19.93	196	Benzofuran	1.11
18	tetradecanoic acid	$C_{14}H_{28}O_2$	20.07	228	fatty acid	0.86
19	neophytadiene	$C_{20}H_{38}$	21.08	278	sesquiterpenoid	0.57
20	n-pentadecanoic acid	$C_{15}H_{30}O_2$	22.66	242	fatty acid	6.95
21	Methyl (7E)-7-hexadecenoate	$C_{17}H_{32}O_2$	24.75	268	Ester	0.75
22	9-octadecenoic acid	$C_{18}H_{34}O_2$	25.02	282	fatty acid	0.35
23	glycerol, 2-palmitate	$C_{19}H_{38}O_4$	29.14	330	fatty acid	0.58
24	squalene	$C_{30}H_{50}$	33.80	410	Hydrocarbon	0.24
25	vitamin E	$C_{29}H_{50}O_2$	38.45	430	Alcohol	0.27
26	stigmast-5-en-3-ol	$C_{29}H_{50}O$	42.54	414	Steroid	3.26
Total						**88.1**

Table 2
Chemical composition of methanol extract of *W. unigemmata* (MEW)

S. No.	Compound	Molecular Formula	Retention Time	Molecular Weight	Nature of Compound	% Contribution
1	ehtyl acetimidate	C_4H_9NO	4.92	87	Ester	3.59
2	2,4 dihydroxy-2,5- dimethyl-3(2H)- furan-3-one	$C_6H_8O_4$	6.65	144	Ketone	0.53
3	4-morpholineacetonitrile	$C_6H_{10}N_2O$	8.87	126	Nitriles	1.35
4	isopentane	C_5H_{12}	9.08	72	Hydrocarbon	0.91
5	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	10.11	144	Ketone	2.77
6	glycerol	$C_3H_8O_3$	10.63	92	Alcohol	7.05
7	dihydroxybenzol	$C_6H_6O_2$	11.54	110	Phenol	1.02
8	5-hydroxymethylfurfural	$C_6H_6O_3$	11.89	126	Furfural	0.93
9	2,4-diacetoxypentane	$C_9H_{16}O_4$	12.10	188	Hydrocarbon	4.56
10	1-Methyl-3,8,9-trioxabicyclo[4.2.1]nonane	$C_7H_{12}O_3$	13.04	144	Hydrocarbon	1.70
11	cyclopentyl alcohol	$C_5H_{10}O$	15.62	86	Alcohol	3.48
12	dihydroactinidiolide	$C_{11}H_{16}O_2$	16.88	180	Terpene	0.32
13	loliolide	$C_{11}H_{16}O_3$	20.32	196	Benzofurans	1.41
14	neophytadiene	$C_{20}H_{38}$	21.08	278	sesquiterpenoid	3.00
15	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	22.20	270	fatty acid	0.92
16	pentadecanoic acid	$C_{15}H_{30}O_2$	22.68	270	fatty acid	9.81
17	phytol	$C_{20}H_{40}O$	24.43	296	Diterpene	3.54
18	glycerol-α-monostearate	$C_{21}H_{42}O_4$	29.13	358	Alcohol	2.58
19	1,2-benzenedicarboxylic acid	$C_{24}H_{38}O_4$	29.41	390	fatty acid	4.87
20	9-hexadecenal	$C_{16}H_{30}O$	32.39	238	Hydrocarbon	1.10
21	squalene	$C_{30}H_{50}$	33.80	410	Hydrocarbon	0.36
22	α-tocospiro	$C_{29}H_{50}O_4$	34.39	462	Alcohol	1.01
23	vitamin E	$C_{29}H_{50}O_2$	38.45	416	Alcohol	7.82
24	β-sitosterol	$C_{29}H_{50}O$	42.56	414	Steroid	17.39
Total						**82.02**

2006), and as a substitute for propylene glycol (Pagliaro and Rossi, 2008).

Similarly, plant phytosterols (β and γ- sitosterols) are well established for their anti-diabetic and cholesterol-lowering properties through *in vitro* and *in vivo* models (Wang and Ng, 1999; Balamurugan et al., 2012; Hwang et al., 2008). Recently, Rui et al. (2017) reported one new kaempferol as well as six other compounds from methanol extract of *W. unigemmata*. However, their absence in all three extract tested in present study can be explained on the basis of chemical variation due to various ecological feature such as temperature, water stress, light condition as well as phenological development (Upadrasta et al., 2011; Abdelgawad et al., 2014). It also explains the substantial differences obtained from previous reports.

Antioxidant activity of phenolics is due to their reducing effect, as hydrogen donor and singlet oxygen quenchers (Chang et al., 2001; Rice-Evans et al., 1997). Polyphenols represent a heterogeneous group of PSMs, which includes flavonoids, phenolic acids, stilbenes, and lignans (Hussain et al., 2016). They are well-documented antioxidant (Hussain et al., 2016) and antibacterial agents (Okwu, 2004; Afolabi et al., 2007). The later been attributed to their iron deprivation or hydrogen bonding ability with

Table 3
Chemical composition of hexane extract of *W. unigemmata* (HEW)

S. No.	Compound	Molecular Formula	Retention Time	Molecular Weight	Nature of Compound	% Contribution
1	4,6-dimethyldodecane	$C_{14}H_{30}$	12.52	198	Hydrocarbon	0.23
2	dihydroactinidiolide	$C_{11}H_{16}O_2$	16.86	180	Terpene	0.59
3	neophytadiene	$C_{20}H_{38}$	21.08	278	sesquiterpenoid	2.49
4	2-hydroxycyclopentadecanone	$C_{15}H_{28}O_2$	22.42	240	Ketone	1.13
5	pentadecanoic acid	$C_{15}H_{30}O_2$	22.79	242	fatty acid	3.90
6	phytol	$C_{20}H_{40}O$	24.43	296	Diterpene	4.38
7	4,8,12,16-tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	27.07	324	Aldehyde	1.21
8	glycerol -α-monostearate	$C_{21}H_{42}O_4$	29.14	358	Alcohol	0.74
9	6,9-*cis*-3,4-epoxy-nonadecadiene	$C_{19}H_{34}O$	32.40	278	Hydrocarbon	0.60
10	α-tocospiro	$C_{29}H_{50}O_4$	34.39	462	Alcohol	1.60
11	γ-tocopherol	$C_{28}H_{48}O_2$	37.07	416	Alcohol	0.58
12	vitamin E	$C_{29}H_{50}O_2$	38.52	430	Alcohol	10.04
13	campesterol	$C_{28}H_{48}O$	40.50	400	Steroid	7.32
14	γ-sitosterol	$C_{29}H_{50}O$	42.84	414	Steroid	33.45
15	cycloartenol	$C_{30}H_{50}O$	44.82	426	Alcohol	4.06
Total						**72.42**

Table 4
Total Phenolic, Flavonoid Content and antioxidant activity (DPPH, FRAP) in different extracts of *W. unigemmata*

Plant extracts	Phytochemicals		Antioxidant activity	
	TPC (mg GAE gm^{-1} dry extract)	TFC (mg QE gm^{-1} dry extract)	FRAP values (mg AAE gm^{-1} dry extract)	DPPH assay (IC_{50} value, μg/ml)
Aqueous extract	363 ± 7.27	146 ± 7.84	605 ± 7.08	50.68 ± 2.5
Methanol extract	873 ± 6.01	151 ± 11.24	768 ± 10.4	6.07 ± 1.4
Hexane extract	31.67 ± 10.94	43.89 ± 1.47	85 ± 5.13	87.27 ± 7.3
***Corr. with TPC**	–	–	0.915	−0.998
***Corr. with TFC**	–	–	0.982	−0.858

Values are mean ± SE of three replicate, TPC- Total Phenolic Content: TFC- Total Flavonoid Content; GAE- Gallic Acid Equivalent; QE- Quercetin Equivalent; AAE- Ascorbic acid Equivalent; *Correlation is significant at the 0.05 level

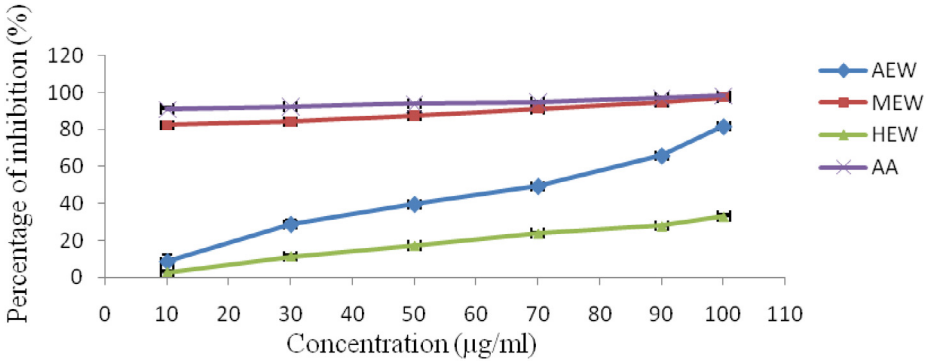

Fig. 1. Antioxidant activity of *W. unigemmata* extracts against DPPH radical; AEW- aqueous extract; MEW- Methanol extract; HEW- Hexane extract; AA-Ascorbic Acid.

Table 5
Antibacterial activity of different extracts of *W. unigemmata* Table 4.

Bacterial strain	Aqueous Extract		Methanol Extract		Hexane Extract		Gentamicin	Ampicillin
	ZOI (mm)	MIC (mg ml^{-1})	ZOI (mm)	MIC (mg ml^{-1})	ZOI (mm)	MIC (mg ml^{-1})	ZOI (mm) at 10mcg/ml	ZOI at 10mcg/ml
RS	8 ± 2.7	20	na	na	15 ± 0.9	30	23 ± 2.3	15 ± 2.3
EC	na	na	na	na	8 ± 2.6	30	21 ± 1.8	7 ± 2.2
XO	na	na	na	na	10 ± 0.7	30	25 ± 1.1	21 ± 0.8
XP	11 ± 0.3	20	na	na	9 ± 0.3	20	22 ± 3.9	16 ± 2.5
XC	10 ± 00	20	na	na	7 ± 00	30	25 ± 1.3	22 ± 1.4
AG	11 ± 0.3	30	8 ± 00	30	8 ± 00	30	22 ± 1.7	16 ± 2.7
KP	na	na	na	na	7 ± 0.3	30	28 ± 1.2	18 ± 1.2
LM	10 ± 3.5	10	na	na	10 ± 3.9	10	21 ± 0.7	na
PM	na	na	na	na	na	na	22 ± 1.2	na
PA	na	na	na	na	na	na	22 ± 1.5	15 ± 5.9

RS- *R. solanacearum*; EC- *E. chrysanthemi*; XO- *X. oryzae*; XP- *X. phaseoli*; XC- *X. compestris*; AG- *A. tumefaciens*; KP- *K. pneumoniae*; LM- *L. monocytogenes*; PM- *P. mirabilis*; PA- *P. aeruginosa*; na- Not active, ZOI- zone of inhibition.

microbial enzymes (Scalbert, 1991; Davidson and Naidu, 2000). Similarly, Cowan (1999) credited the complex-forming ability of flavonoids with the microbial cell wall and soluble proteins, which makes them responsible for antimicrobial activity.

The antioxidant activity and TPC in the three samples were in the order of MEW > AEW > HEW, which demonstrates the influence of solvents extractability. Several reports have shown that the nature of the solvent exerts a significant influence on the phenolic extraction of plants (Akowuah et al., 2004; Turkmen et al., 2006). Further, MEW and AEW maintained high antioxidant activity, which is comparable in both total DPPH and FRAP assays. This observation suggests that chemical/s responsible solitarily or synergistically can be extracted in polar solvents in much higher amount. Further, these chemical/s can function using two dissimilar machinery, for free radical scavenging activity, through a single electron transfer in FRAP (Ashby, 1988), and as hydrogen transfer reaction for DPPH activity (Prior et al., 2005; Mader et al., 2007; Shalaby and Shanab, 2013).

We observed a positive correlation between total phenolic content and antioxidant activities (Table 4) in a dose-dependent manner, which affirm several previous reports (Velioglu et al., 1998; Mei et al., 2009; Pandey et al., 2019). Previously, Zhong et al., (2008), also reported high DPPH scavenging activity (IC_{50}-7.20 μg/ml) of ethanol extract of *W. unigemmata*. The proximity of polarity might be a possible explanation for similar findings between methanol and ethanol. Interestingly, HEW, which had low antioxidant activity, exhibited higher broad-spectrum antibacterial activity than AEW and MEW. This variation could be explained based on a high amount of γ-sitosterol (33%) alone or synergistically affecting microbial growth. Further, the docking capacity of certain molecules (γ-sitosterol, in this case) with target protein might be responsible for hindering biosynthetic pathways of microorganisms. These results are of enormous value, mainly in the view of growing drug resistance among bacteria.

These findings of the present study are promising and actively encouraging for further research, such as fractionation of extracts and confirmation of mechanism involved. To the best of the facts known and our understanding, this is the pioneer report on the chemical characterization together as well as the antioxidant and antibacterial activity of any fern species from the Indian Himalayan region.

5. Conclusion

The present study reports the presence of many medicinally valuable and industrially relevant compounds, including glycerol, catechol, tocopherols, phytosterols, etc. in *W. unigemmata.* Tested plant extracts showed remarkable antibacterial activity against plant as well animal pathogenic bacteria. In addition, they are recorded with rich polyphenolic compounds (TPC and TFC) with efficient antioxidant activity in both, DPPH, and FRAP assays. Based on these observations, *W. unigemmata* can be recommended as a source of many biologically active and industrially relevant compounds. Further, research on individual compound is required to assess their potential, especially in the pharmacology sector.

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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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.06.006.

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