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Antioxidant and antimicrobial activity and GC-MS analysis of extract of *Rumex nepalensis* Spreng

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Abstract
Rumex nepalensis Spreng. Subjected to extraction with methanol, hexane, chloroform and ethyl acetate revealed the presence of alkaloids, flavonoids, glycosides and steroids from the phytochemical screening. The antibacterial, antifungal and antioxidant activities of the crude ethyl acetate extract of *R. nepalensis* were studied. The ethyl acetate fraction showed inhibitory effect against *Staphylococcus aureus* and *Escherichia coli* with the MIC values of 6.3 mg/mL and 3.1mg/mL, respectively. It failed to show any inhibition against the tested fungi. While the crude ethyl acetate extract showed efficient DPPH antioxidant activity with IC_{50}± SEM = 26.6 ± 3.1 µg/mL. The above selected plant was shown by *in vitro* assays to be a potential source for natural antioxidant and antibacterial agent. GC-MS analysis showed the presence of 20 major possible compounds.

Keywords: *Rumex nepalensis* Spreng, ethyl acetate extract, antioxidant and antibacterial activity.

Introduction
Rumex nepalensis Spreng. Belongs to the family polygonaceae and called as 'Halhale' in Nepali. It is widely distributed throughout Himalayas from Bhutan to Kashmir and also to Turkey, Java and South Africa. It is fairly common plant of higher altitudes and grows between 900-4300 m on moist as well as dry slopes, under shades and even in plains throughout Nepal's hilly region. *R. nepalensis* is perennial ascending herbs having tap roots and erect stems which is 50-100 cm tall. Its leaves structure is basal and petiole is 4-10 cm wide. Leaves entire, lower ones long-stalked, oblong, ovate, petiole, base widely or narrowly cordate. Flowers bisexual, in whorls forming long, nearly leafless racemes, reddish or green [1]. The presence of new secoanthraquinone glucosides, nepalensides and some known compounds such as torachrysone, rumexoside, orientaloside, orcinol glucoside, aloesin, lyoniresinol 3*α*-O-*β*-D-glucopyranoside, (-)-epicatechin-3-O-gallate, (3,5-dimethoxy-4-hydroxyphenol)-1-O-β-D-(6-O-galloyl) glucose, and (-)-epicatechin gallete have been previously reported in the root of *R. nepalensis*. Determination of chrysophanol-8-O-*β*-D-glucopyranoside and nepodin were detected as major constituents of anthraquinone and naphthalene derivatives in *R. nepalensis* roots by HPLC. The root of *R. nepalensis* is purgative, also used against venereal diseases and bilharziasis. According to the previous investigation root contains Chrysophanol, Physcion, Leupeol, *β*-sitosterol and its glucoside, Orientalone, emodin, 1-O-*β*-D-glucopyransylmusizin, 3-methoxy-5,6-methylenedioxy benzaldehyde and chrysophanol-8-O-*β*-D-galactopyranoside [2, 3].

R. nepalensis plays a vital role in traditional medicine. Nearly all parts of plants are used as traditional medicines in different part of the world. Like ways, *R. nepalensis*, used for various therapeutic purpose is well known traditional ayurvedic medicine in Nepal as an anti-oxidant, analgesic, antipyretic, anti-inflammatory, anti-diarrhoeal, anti-hypertensive, and psychopharmacological activities. It's leaf extract is applied to cure skin sores and also leaf is applied to syphilitic ulcers and the treatment of colic and headaches. It's aqueous extract also use as wash for reducing body pain. The root is purgative. The root is applied to dislocated bones. Also, paste of the root is applied to swollen gums and for treatment of diarrhoea and dysentery. In some Nepalese villages, it is used as green leafy vegetable also.

Experimental
Collection of Plant Materials
The plant was collected from Regional Agriculture Research Centre, Lumle, Kaski, Nepal. The collected plants were identified by Prof. Dr. Devendra M. Bajracharya, Department of Botany, Amrit Campus.

Preparation of Plant Extracts
The dried plant (520 g) was grinded to powder and further proceeded via cold percolation process for 5 days for three times with 2.5 liters methanol the process was repeated three times and concentrated by Rota-vapor. The extract (35 g) was fractionated into hexane, chloroform, and ethyl acetate.

GC-MS Spectra Analysis
GC/MS analysis was performed on a gas chromatography mass spectrometer GCMS-QP 2010 under the following condition: injection volume 1µL with split ratio 1: 90; Helium as a carrier gas with a Rtx-5MS column of dimension 30m× 0.25mm×0.25 µm, temperature programmed at 50, 150 and 250 °C with a hold time of 0.0, 2.0 and 5.0 min identification was accompanied by comparison of MS with those reported in NIST 05 and FFNSC 1.3 libraries (The National Institute of Standards and Technology is a measurement standards laboratory and a non-regulatory agency of the United States to promote innovation and industrial competitiveness). The test method was ISO 7609: 1985. The GC-MS analysis was carried out in National Forensic Science Lab, Khumaltar, Kathmandu.

Antioxidant Activity Assay
Firstly, 1 mg of the sample to be tested was dissolved in 1 ml methanol to get stock solution of concentration 1 mg/ml. 100 µl of these solutions were added to 100 µl of 0.1mM DPPH (0.4 mg DPPH in 100 ml methanol) differently and was left for 30 minutes in dark room. After 30 minutes, the samples like the color of DPPH, which do not show antioxidant property was discarded and for the sample with the yellow color (more than 50% inhibition then control) was taken for further testing as they were expected to be the potential antioxidants.
Different concentrations of the 100 µl extracts (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 µg/ml) were prepared by two-fold dilution method and 100 µl of 0.2mM DPPH solution was added. The solutions were kept in dark for 30 minutes and their absorbance was taken at 517 nm against DPPH as a blank. Sample was omitted for control. Quercetin of same concentration was prepared as standard and its absorbance were also taken spectrophotometrically at 517 nm. And a calibration curve was prepared.

Antimicrobial activity
The single colony was adjusted to 0.5 McFarland units (about 1×10^8 CFU/mL) by 1-4 hours incubation in nutrient broth (NB) and seeded all over the in the surface of Mueller Hinton agar (MHA) and left to dry for few minutes at room temperature. Then wells (6 mm) were made by a sterile cork borer and 50 µL of the plant extract (25 mg/mL) and only DMSO (50%) and neomycin as a control were loaded into the respective wells then left for 30 min and incubated at 37°C for 18-24 hours to observe the zone of inhibition [4].The zone of inhibition is the clear area around the well and average of the diameters of zone of inhibition of triplicate was taken.

Determination of Minimum Bactericidal Concentration (MBC)
The crude extract was two folds serially diluted in NB media (25-0.078 mg/mL) and along with positive and negative control containing only NB. Then 20 µL of inoculums from 0.5 McFarland (1×10^8 CFU/mL) was added to all tubes except negative control. After 24 hours incubation at 37°C, all the tubes were sub-cultured on antimicrobial free Nutrient agar plates and incubated at 37°C for 24 hours. MBC values were the first tube that showing no growth on solid media.[4]

Antifungal Assay
A single pure colony of the fungus was inoculated in Potato dextrose broth (PDB) and incubated at 28°C for 8-24 hours to gain 0.5 McFarland units while shaking. This inoculum (150 µL) was seeded all over the surface of Potato dextrose agar (PDA) by sterile bent glass rod and left to dry for few minutes at room temperature. Then wells (6 mm) were made by a sterile cork borer and 50 µL of the crude extract (25 mg/mL) and only DMSO (blank) as a control were loaded into the respective wells then left for 30 min and incubated at 28°C for 3-6 days to observe [5].

Result and Discussion
The composition of Ethyl-acetate extract of *Rumex nepalensis* was analyzed by GC-MS coupled with mass library search (NIST 11, FFNSC 1.3) revealed the presence of 20 major compounds. Major compounds found by GC-MS analysis were shown below.

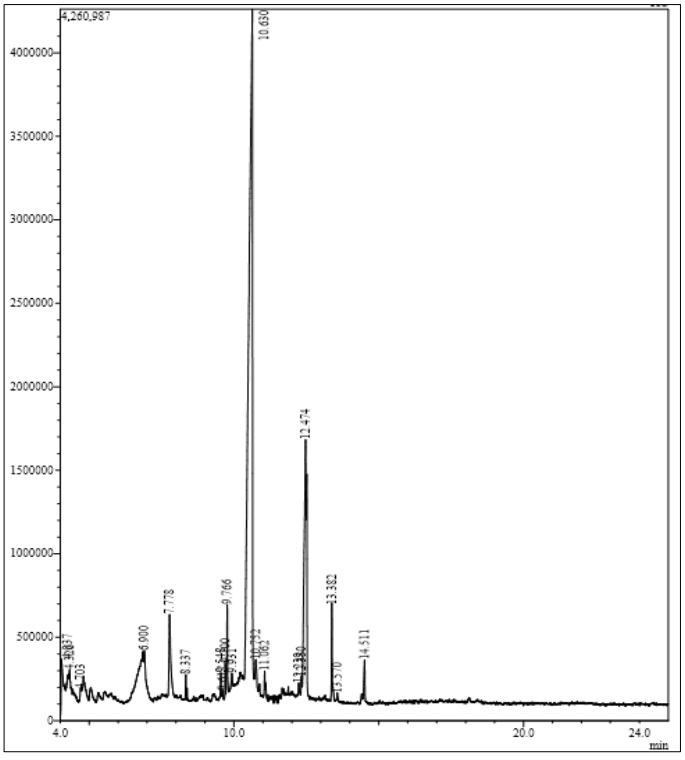

Fig 1: Chromatogram of ethyl-acetate extract of *R. nepalensis*

Table 1: List of compounds detected in ethyl-acetate extract of *R. nepalensis*

S. No	Name of the compound	Retention time (min)	Molecular formula	Molecular weight	Area (%)
1.	(vinyloxy) benzene	4.037	C_8H_8O	120	1.97
2.	Benzeneacetic acid	4.326	$C_8H_8O_2$	136	0.75
3.	Indole	4.703	C_8H_7N	117	0.25
4.	(1-iodo) Tridecane,	6.900	$C_{13}H_{27}I$	310	0.55

5.	p-Terphenyl	7.778	$C_{18}H_{14}$	230	3.76
6.	1-Octadecene	8.337	$C_{18}H_{36}$	252	0.36
7.	Pentadecanoic acid	9.574	$C_{15}H_{30}O_2$	242	0.48
8.	bis(2-methylpropyl)ester 1,2-Benzenedicarboxylic acid	9.617	$C_{16}H_{22}O_4$	278	0.17
9.	1-Octadecene	9.700	$C_{18}H_{36}$	252	0.45
10.	1z,2z-1,2-bis(3,5,5-trimethylcyclohex-2-en-1-ylidene)hydrazine	9.766	$C_{18}H_{28}N_2$	272	1.98
11.	7-hydroxy-2,5-dimethyl 4H-1-Benzopyran-4-one	9.931	$C_{11}H_{10}O_3$	190	0.25
12.	1-(1-hydroxy-3-methoxynaphthalen-2-yl)ethan-1-one	10.630	$C_{13}H_{12}O_3$	216	65.08
13.	cis-Vaccenic acid	10.752	$C_{18}H_{34}O_2$	282	1.20
14.	(E)-3-Ecosene	11.062	$C_{20}H_{40}$	280	0.82
15.	(3-methyl)Anthracene-1,8,9-triol	12.239	$C_{15}H_{12}O_3$	240	0.43
16.	1-Tricosene	12.330	$C_{23}H_{46}$	322	0.51
17.	1-Iodo-2-isopropylbenzene	12.474	$C_9H_{11}I$	246	17.36
18.	bis(6-methylheptyl)phthalate	13.382	$C_{24}H_{38}O_4$	390	2.25
19.	1-Tricosene	13.570	$C_{23}H_{46}$	322	0.27
20.	1,8-dihydroxy-3-methoxy-6-methyl-4a,9a-dihydroanthracene-9,10-dione	14.511	$C_{16}H_{12}O_5$	284	1.09

Table 2: Antibacterial activity of the ethyl acetate fraction

Sample	**Zone of Inhibition* (mm) and (MIC (mg/mL))**			
	EC	***SA***	***ST***	***KP***
extract	22 (3.1)	18 (6.3)	-	-
Neomycin**	24	22	14	23

***Diameter of well = 6 mm
****Control antibiotics
SA = *Staphylococcus aureus* ATCC 25923
ST = *Salmonella entericaserovarTyphimurium* ATCC 14028
EC = *Escherichia coli* ATCC 25922
KP = *Klebsiellapneumoniae* ATCC 700603

Table 3: Antifungal activity of the ethyl acetate fraction.

Sample	Zone of Inhibition* (mm)				
	Fusarium spp.	*Trichoderma spp*	*A. niger*	*A. flavus*	*Rhizopusspp.*
1	-	-	-	-	-
cyclohexamide**	32	28	25	32	34

***Diameter of well = 6 mm
****Control antibiotics

Antioxidant screening analysis

The antioxidant capacity of plants is clearly associated with the activity of "free radical scavenging enzymes" (superoxide dismutase, catalase, peroxidase, etc.) and the contents of antioxidant substances mainly phenolic compounds, carotenoids, tocopherol and ascorbic acid. The antioxidant potential is in an inverse relation with IC_{50} value, which can be calculated from linear regression of the % inhibition verses antioxidant activity. Lower the IC_{50} value indicates high antioxidant activity. All the calculations are based on the standard method [6]. Absorbance was measured at 517 nm.

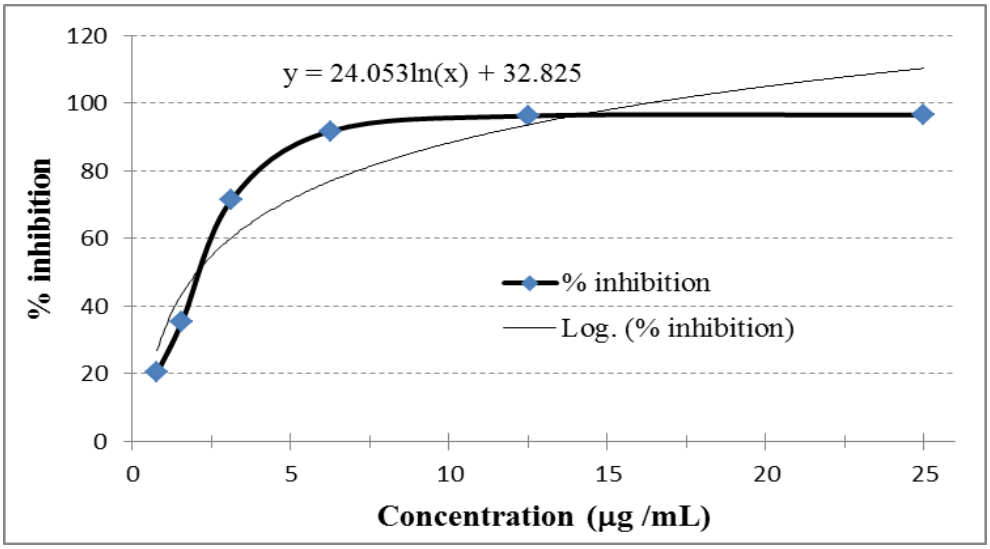

Fig 2: Graphical representation of DPPH assay of the extract The IC_{50} ± SEM of this ethyl acetate fraction was found to be 26.6 ± 3.1 µg/mL and the standard, quercetin was 2.3 ± 0.1 µg/mL.

Conclusion

Phytochemical Screening analysis of ethyl acetate extract indicates the presence of Flavonoids, Glycosides, Steroids, Sugar and Quinones. Ethyl acetate fraction showed high potential of antibacterial activity for both gram positive (*S. aureus*) and gram negative (*E. coli)* bacteria. DPPH assay and IC_{50} value determination showed that ethyl acetate fraction of *Rumex nepalensis* has $IC_{50} \pm$ SEM equal to 26.6 ± 3.1 µg/mL. and GC-MS analysis showed the presence of 20 major possible compounds.

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