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Chemical Constituents and Biological Activities of Ginger Rhizomes from Three Different Regions of Nepal

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Abstract

We aimed to establish the chemical constituents of essential oil and biological assays from crude ethanolic extract of ginger from Sindhupalchhowk, Tanahu and Gorkha region of Nepal. Ginger essential oils were extracted using hydro distillation, and having the yield percent; Gorkha: 1.9, Sindhupalchowk: 1.8 and Tanahu: 1.1. GC-MS analysis revealed the presence of 105, 85, 88 components from Sindhupalchowk, Tanahu and Gorkha ginger respectively, with the major constitutes, monoterpenes and sesquiterpenes derivatives. The Soxhlet extraction was performed for the 95 % ethanolic extraction from fresh ginger (EE) and the residue ginger after essential oil extraction (EEROE). The antimicrobial activity of 8% EE & EEROE against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Klebsiella spp*. was expressed in zone of inhibition (mm) and comparable with chloramphenicol Similarly, the antioxidant assay was performed by using DPPH radical scavenging method in different concentration. Where, EE & EEROE showed the maximum percentage inhibition as compared with ascorbic acid but the essential oil did not possess the antioxidant activity.

Keywords: Ginger; GC-MS, Antioxidant; Essential oil; Ethanolic extract; DPPH radical scavenging

Abbreviations: EE: Ethanolic extraction from fresh ginger EEROE: Ethanolic extract of residue after oil extraction; DDPH: 2,2-diphenyl-1-picrylhydrazyl; AITM: Asian Institute of Technology and Management

Introduction

Ginger (*Zingiber officinale Roscoe*), belonging to the family Zingiberaceae. It is grown throughout the tropical areas of the world and also commonly found in South East Asia especially in Indo-Malaysia. Ginger is the topmost spice crop both in terms of area of cultivation and production volume, traditionally grown in the mid-hill areas of Nepal [1]. According to the FAO, 2014, Nepal is third in worldwide ginger production after India and China [2]. The health benefit properties of ginger has been documented more than 2000 years ago [3] and has been used in Ayurvedic and Unani systems of medicine in Indian and Arabic herbal traditions since ancient times, for different diseases [4]. Now, the ginger has widely been used for the treatment of various diseases, including

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gather osclerosis, migraine headaches, rheumatoid arthritis, high cholesterol, ulcers, depression, and impotence [5]. Numerous active ingredients are present in ginger ranging from volatile components which comprise approximately 1-3% of its weight [6] to non-volatile components.The major active ingredients in ginger oil are thesesquiterpenes (30-70%): bisapolene, zingiberene, and zingiberol [7,8]. The active chemical constituents in the ginger are phenolic compounds: shogaols and gingerols, Sesquiterpenes: bisapolene, zingiberene, zingiberol, sesquiphellandrene, curcurmene and other compounds like 6-dehydrogingerdione, galanolactone, gingesulfonic acid, zingerone, geraniol, neral, monoacyldigalactosylglycerols, gingerglycolipids [9,10]. The pungent odor in the ginger is due to the gingerols. We, therefore in continuation of our research in phytochemical and biological screening, this time we have chosen the ginger from the three major areas for cultivation of Nepal, i.e., Sindhupalchowk, Tanahu and Gorkha, in a hope to find out the chemical constituents of ginger oil and ethanolic extracts and compare with its biological activities like antimicrobial and antioxidant activity.

Method and Materials

Collection of the Sample

The fiber-less ginger was collected from three different places of Nepal, Sindhupalchowk (Latitude: 27.77, Longitude: 85.70), Tanahu (Latitude: 27.91667, Longitude: 84.25), and Gorkha (Latitude: 27.97, Longitude: 84.60). The rhizome part of the ginger was washed, chopped, air dried and grinned for the essential oil and 95% ethanolic extraction.

Hydro distillation

In order to extract the essential oils, 100 g of the powder was placed in 1 liter conical flask and connected to the Clevenger apparatus. 500 mL of distilled water was added to the flask and heated to the boiling point. The steam in combination with the essential oils were distilled in to a graduated cylinder for 3 hours and then separated from aqueous layer and repeated three times. The oil was refrigerated until further analysis and the remaining residue of ginger after essential oil extraction was taken for the further ethanolic extraction (EEROE) [11].

Soxhlet -ethanolic extraction

The dried ginger samples (fresh ginger and residue of ginger after essential oil extraction) were grinded into fine powder. About 80g of powdered sample was exhaustively extracted with 95% ethanol using soxhlet apparatus for about 13 hours. The residue was concentrated using rotavapour to obtain solvent free extracts and stored in refrigerator until further use.

GC-MS Analysis

The essential oils were subjected for the gas chromatography–mass spectrometry (GC–MS), using a Shimadzu GC-MS model GC-2010 equipped with Mass spectrophotometer GC-MS QP 2010 with the following parameters mentioned in Table 1.

GC-MS conditions	Parameters	
Column oven temperature	50 ^{0}C	
Injection temperature	200 ^{0}C	
Injection mode	Split	
Flow control mode	Linear velocity	
Pressure	120 kPa	
Total flow	185.7 mL/min	
Column flow	2.04 mL/min	
Linear velocity	51.9 cm/sec	
Purge flow	0.1 mL/min	
Split ratio	90	
	Temperature (^{0}C)	Hold time (minute)
-	50	0
4	140	0
11	206	0
14	250	9

Table 1: Gas Chromatography-Mass Spectrometer (GC-MS).

Phytochemistry

Phytochemical screening of the crude ethanolic extract of fresh-dried ginger (EE) and the residue ginger after essential oil extraction (EEROE) was carried out using standard phytochemical methods as described previously [12,13].

Antimicrobial screening

Agar- well diffusion method: Eight % crude EE & EEROE from Sindhupalchowk, Tanahu and Gorkha of Nepal were tested for their antibacterial property against four pathogenic bacteria: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsiella spp*. These microbes were preserved and maintained at Asian Institute of Technology and Management (AITM), Lalitpur, Nepal. The zone of inhibition was measured by agar well method (6mm diameter) using Muller Hinton Agar medium. The zone of inhibition was compared with Chloramphenicol antibiotic as standard.

Antioxidant assay: The antioxidant activity of ginger oil and EE & EEROE were carried out by DPPH free radical

scavenging assay using the method of Liyana- Pathiranan and Shahidi [14]. The different concentrations of extracts were added to 10ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30min at room temperature in dark place. Changes in absorbance of samples were measured at 517nm. Ascorbic acid was used as the standard control. All the tests were performed in triplicates. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula [15].

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_s) \times 100]/A_0$$

Where, A_0 is absorbance of the blank, and A_s is absorbance of the samples at 517 nm.

Statistical Analysis

Data are expressed as means ± standard error (SEM) and statistical analysis was performed with Microsoft excel 2013. Difference on statistical analysis of data were considered significant at $P<0.05$.

Results and Discussion

Extraction yield

The essential oil extracted from Gorkha (1.9%) and Sindhupalchowk (1.8 %) had higher yield ratio than the essential oils from Tanahu (1.1 %). Ginger contains 1-3.3 % oil by steam distillation [16]. In our current study, the percentage yield of essential oil from Sindhupalchowk, Gorkha and Tanahu lies between the above mentioned values, but the difference in the yields might be due to loss of peel of rhizomes during transportation. As the means of transportation was varied due to the geographical verification of the sampling collecting site. The rhizome peel is responsible for oil content. The fiber less ginger got charred easily when the ratio of finely grinded ginger and water was not properly made. This resulted change in odor of essential oils. But in case of fiber ginger the froth formation was the main problem that might cause the impurities of the essential oils. Significant amount of yields was obtained from the ethanol extraction. The ethanolic extracts of Sindhupalchowk and Gorkha were found to have higher yield when compared with the extract obtained from Tanahu, in both samples, EE and EEROE (Table 2). The geographical area of cultivation, time of harvest and nature of ginger cultivars might affect the amount of yield. Similarly, yield percentage of extract is dependent on time length, because a longer extraction time will have high chance of contact between the solvent and material [17].

Ginger sample	Essential oil	Ethanolic-Extract	
		EEROE	EE
	Product Yield %		
Sindhupalchok	1.8	9.3	13.3
Tanahu	1.1	2.4	6.3
Gorkha	1.9	8.5	9.7

Table 2: Extraction yield of ginger from Sindhupalchok, Tanahu and Gorkha.
EE: Ethanolic extract
EEROE: Ethanolic extract of residue after oil extraction

Phytochemical screening of ethanolic extract & GC-MS analysis of essential oil

Phytochemical screening of the ethanolic extraction of EE and EEROE from Sindhupalchowk, Tanahu and Gorkha revealed the presence of biologically active constituents like Glycosides, flavones, reducing sugars, steroids and terpenoids (Table 3). Alkaloids, saponins, tannins, coumarins were not detected from ethanolic extracts. However, many studies reported the presence of alkaloids, tannins, saponins from the chloroform, methanol and ethanol extract of crude ginger [18-20].

Phytochemical	S-EE	S-EEROE	T-EE	T-EEROE	G-EE	G-EEROE
Basic alkaloids	-	-	-	-	-	-
Glycosides	++	+	+	++	++	++
Saponins	-	-	-	-	-	-
Flavones	++	+	++	+	++	+
Reducing sugars						
A. Fehling's test	+	++	++	+	++	++
B. Benedict's test	++	++	++	+	++	++
Tannins & polyphenols	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-
Diterpenes	++	++	++	+	++	+
Phlobatanin	-	-	-	-	-	-
Steroids & terpenoids	++	++	++	++	++	++

Table 3: Phytochemical screening of Ethanolic extract of ginger from Sindhupalchok, Tanahu, and Gorkha.
(++) test strongly positive, (+) weak positive test, (-) negative test (absence of required ppt/color) S: Sindhupalchok, T: Tanahu and G: Gorkha
EE: Ethanolic extract
EEROE: Ethanolic extract of residue after oil extraction

To determine the components of the essential oil, GC-MS was performed and 105, 85 and 88 peaks were detected from the essential oils of Sindhupalchowk, Tanahu and Gorkha respectively (Table 4a,4b,4c). Among those the

major constituents, α-Funebrene, α-Farnesene, β-Phellandrene, Sesquisabiene, Camphene, α -Pinene, Linalool, Borneol, Geraniol, Geranial, Neral, Citronellol, Aryl-curcumene were detected from ginger oil of three regions. The content of α-Funebrene was found to be higher percentage in essential oil from Sindhupalchowk (12.5 %), Tanahu (10.4 %) and Gorkha (12.5 %). Methylheptenonewas highly detected in ginger oil of sindhupalchowk (8.59) when compared with Tanahu (1.15) and Gorkha (1.63). However, Geranyl acetate was not detected in Tanahu ginger. Interestingly, the major constituents in the ginger oil, Zingiberene, Curcumene and Citral were not detected in the ginger oil from Sindhupalchok, Tanahu and Gorkha.

Peak	R time (min)	Compounds	Amount %
1	4.484	2-Heptanone	0.22
2	4.687	2-Heptanol	0.53
3	5.228	Tricyclene	0.17
4	5.54	α-Pinene	2.11
5	5.963	Camphene	4.19
6	6.563	Sabinene	0.2
7	6.655	β-Pinene	0.31
8	7.09	Methylheptenone	8.59
9	7.462	α-Phellandrene	0.63
10	8.099	O-cymol	0.15
11	8.31	β-Phellandrene	8.03
12	8.597	acetic acid, sec-octyl ester	0.16
13	9.091	Oct-(2E)-enal	0.11
14	9.504	1-Octanol	0.13
15	10.067	Terpinolene	0.31
16	10.194	2-Nonanone	0.47
17	10.353	2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-octahydro-4a-methyl-	0.14
18	10.49	Linalool	1.44
19	11.161	trans-2-Pinanol	0.13
20	11.93	Camphore	0.18
21	12.055	Camphene hydrate	0.12
22	12.227	(R)-(+)-Citronellal	0.39
23	12.697	Borneol	1.34
24	13.041	Terpinen-4-ol	0.2
25	13.23	Isogeranial	0.16
26	13.362	Cryptone	0.16
27	13.52	α-Terpineol	0.7
28	13.702	Myrtenol	0.12
29	13.984	n-Decanal	0.13
30	14.602	2,3-epoxygerianial	0.1
31	14.919	Citronellol	2.59
32	15.104	2-Acetoxytridecane	0.15
33	15.33	Neral	3.53
34	15.617	trans-Chrisanthenyl acetate	0.12
35	15.801	Geraniol	2.15
36	15.893	(2E)-decenal	0.2
37	16.361	Geranial	4.56
38	16.732	Bornyl acetate	0.36
39	16.973	2-Undecanone	0.96
40	17.184	2-Undecanol	0.13
41	18.358	δ-elemene	0.12
42	18.882	Citronellyl acetate	0.38
43	19.225	Cyclosativene	0.28

44	19.605	Copaene	0.45
45	19.881	Geranyl acetate	0.8
46	20.061	α-Cubene	0.1
47	20.126	β-Elemene	0.31
48	20.547	α-Fenebrene	0.27
49	20.986	(+)(E)-Caryophyllene	0.12
50	21.48	α-Bergamotene	0.16
51	21.897	α-Guaiene	0.18
52	22.143	trans-β-bergamotene	0.69
53	22.281	9-epi-Caryophyllene	0.36
54	22.732	Selina-4,11-diene	0.64
55	22.939	unknown	2.34
56	23.048	Aryl-curcumene	3.95
57	23.496	α-Funebrene	12.46
58	23.572	α-Muurolene	0.28
59	23.739	α-Farnesene	7.27
60	23.936	unknown	0.32
61	24.105	Sesquisabinene	5.73
62	24.188	(E)-γ-Bisabolene	0.4
63	24.354	4a(2H)-Naphthalenecarboxylic acid, 1,3,4,5,6,7-hexahydro-1,1-dimethyl-2-oxo, ethyl ester	0.2
64	24.522	3,7-Cyclodecadiene-1-methanol,.alpha,.alpha,.4,8-tetramethyl-,[s-(Z,Z)]	0.27
65	24.581	7-epi-trans-Sesquisabinene hydrate	0.36
66	24.71	Germacrene B	0.93
67	24.755	1,6,10-Dodecatrien-3,7,11-trimethyl-, (E)-	0.65
68	25.015	Germacrene D-4-ol	0.14
69	25.072	Cadin-4-en-10-ol	0.13
70	25.25	7-epi-trans-Sesquisabinene hydrate	0.58
71	25.405	Humulane-1,6-dien-3-ol	0.23
72	25.527	Cubenol	0.2
73	25.656	α-(−)-bisabolol	0.94
74	25.793	γ-Eudesmole	0.42
75	25.92	Epiglobulol	0.97
76	26.035	unknown	0.11
77	26.106	Viridiflorol	0.34
78	26.16	Aryl-curcumene	0.13
79	26.269	β-Eudesmol	0.47
80	26.316	Cadin-4-en-10-ol	0.3
81	26.395	unknown	0.3
82	26.506	7-epi-trans-Sesquisabinene hydrate	0.33
83	26.591	Valeranone	0.22
84	26.775	Carotol	0.96
85	26.872	α-Springene	0.62
86	27.111	Bicyclo[10.1.0]trideca-4,8-diene<13-oxa-,trimethyl->	0.75
87	27.187	Z,Z-Farnesol	0.22
88	27.3	Nuciferol	0.7
89	27.379	Dehydronerolidol	0.26
90	27.45	E,E-Farnesol	0.47
91	27.588	α-trans-Bergamotenol	0.52
92	27.689	unknown	0.13
93	27.744	Caryophyllene oxide	0.26

94	27.914	1,2-Oxaborole,2,3,4-triethyl-2,5-dihydro-5,5-dimethyl-	0.19
95	28.059	2,5,5,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	0.52
96	28.268	Cyclohexane, 3,4-bis(1-methylethenyl)-1,1-dimethyl-	0.44
97	28.362	Bicyclo[10.1.0]trideca-4,8-diene<13-oxa-,trimethyl->	0.11
98	28.475	unknown	0.1
99	28.728	trans-Longipinocarveol	0.12
100	28.928	Acorenone 1	0.39
101	29.048	5,9-Undecadien-2-ol,6,10-dimethyl-	0.12
102	29.168	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	0.27
103	29.328	Corymbolone	0.32
104	34.815	4-(2,2-Dimethyl-6-methylenecyclohexyl)butanal	0.15
105	35.698	Hexadeca-2,6,10,14-tetraen-l-ol, 3,7,11,16-tetramethyl-, (E,E,E)-	0.19
Total area			**100**

Table 4a: Chemical composition of ginger oil from Sindhupalchok analyzed by GC-MS.
R. time: Retention time

Peak	R. Time	Compounds	Amount %
1	5.164	Menthol	0.73
2	5.222	Tricyclene	0.37
3	5.528	α-Pinene	2.54
4	5.951	Camphene	6.33
5	6.165	Methyl-3-aminobenzoate	0.18
6	6.554	Sabinene	0.23
7	6.645	β-Pinene	0.42
8	6.935	Methylheptenone	1.15
9	7.048	Myrcene	1.59
10	7.422	γ-Terpinene	1.59
11	7.6	δ-3-Carene	0.2
12	8.045	unknown	0.29
13	8.243	β-Phellandrene	6.67
14	8.293	Eucalyptol	1.77
15	9.072	Oct-(2E)-enal	0.21
16	10.054	Terpinolene	0.34
17	10.177	2-Nonanone	0.23
18	10.334	Phellandral	0.22
19	10.448	Linalool	1.06
20	11.148	trans-2-Pinanol	0.21
21	11.919	Camphore	0.27
22	12.045	Camphene hydrate	0.18
23	12.216	(R)-(+)-Citronellal	0.41
24	12.698	Borneol	2.7
25	13.033	Terpinen-4-ol	0.31
26	13.218	Isogeranial	0.19
27	13.343	Cryptone	0.2
28	13.501	α-Terpineol	0.79
29	13.688	Myrtenal	0.33
30	14.595	2,3-epoxygerianial	0.31
31	14.839	Citronellol	1.15
32	14.946	2,3-epoxygerianial	0.41
33	15.298	Neral	5.17

34	15.72	Geraniol	1.35
35	15.867	(2E)-decenal	0.23
36	16.331	Geranial	6.82
37	16.716	Bornyl acetate	0.74
38	16.935	2-Undecanone	0.46
39	18.864	Citronellyl acetate	0.19
40	19.241	Cyclosativene	0.26
41	19.592	Copaene	0.49
42	19.853	3,7-dimethylocta-2,6-dienyl acetate	0.49
43	20.112	β-Elemene	0.45
44	20.533	α-Fenebrene	0.27
45	21.78	(E)-β-farnesene	0.29
46	22.125	trans-β-bergamotene	0.76
47	22.264	9-epi-Caryophyllene	0.37
48	22.71	α-Selinene	0.58
49	23.02	Aryl-curcumene	8.2
50	23.38	α-Funebrene	10.41
51	23.471	α-Muurolene	0.23
52	23.65	α-Farnesene	7.53
53	23.863	(-)-α-Panasinsen	0.25
54	24.03	Sesquisabinene	6.43
55	24.137	(E)-γ-Bisabolene	0.37
56	24.3	1.α.,10.α.-Epoxy-amorph-4-ene	0.25
57	24.488	unknown	0.29
58	24.555	7-epi-trans-Sesquisabinene hydrate	0.42
59	24.667	Germacrene B	0.61
60	24.733	1,6,10-Dodecatrien-3,7,11-trimethyl-, (E)-	1.01
61	24.958	Cadin-4-en-10-ol	0.65
62	25.055	Cadin-4-en-10-ol	0.47
63	25.233	7-epi-trans-Sesquisabinene hydrate	0.77
64	25.389	Humulane-1,6-dien-3-ol	0.19
65	25.514	unknown	0.18
66	25.637	α-(−)-bisabolol	1.05
67	25.775	unknown	0.5
68	25.904	7-epi-trans-Sesquisabinene hydrate	1.18
69	26.092	Viridiflorol	0.46
70	26.253	β-Eudesmol	0.41
71	26.3	Cadin-4-en-10-ol	0.3

72	26.375	unknown	0.3
73	26.495	7-epi-trans-Sesquisabinene hydrate	0.28
74	26.585	Viridiflorol	0.81
75	26.648	AC1NSY23	0.18
76	26.759	Carotol	0.89
77	26.857	α-Springene	0.51
78	27.1	isoaromadendrene epoxide	0.49
79	27.288	Benzene, 1-(3-cyclopentylpropyl)-2,4-dimethyl-	0.36
80	27.366	Dehydronerolidol	0.2
81	27.438	Farnesal	0.22
82	27.559	unknown	0.31
83	27.731	unknown	0.25
84	28.041	Geranyl linallol	0.25
85	28.249	Bicyclo[10.1.0]trideca-4,8-diene<13-oxa-,trimethyl->	0.29
Total area			**100**

Table 4b: Chemical composition of ginger oil from Tanahu analyzed by GC-MS.
R. time: Retention time

Peak	R. Time	Compounds	Amount %
1	5.147	2-Heptanol	0.23
2	5.715	Tricyclene	0.21
3	6.055	α-Pinene	2.69
4	6.507	Camphene	6.75
5	7.257	β-Pinene	0.63
6	7.61	Methylheptenone	1.63
7	7.722	Myrcene	1.6
8	7.773	6-Hepten-1-ol, 2-methyl-	0.24
9	8.097	α-Phellandrene	0.61
10	8.76	unknown	0.16
11	8.965	β-Phellandrene	5.12
12	9.026	Eucalyptol	4.43
13	9.763	Menthol	0.28
14	9.851	Oct-(2E)-enal	0.16
15	10.865	Terpinolene	0.38
16	11.005	2-Nonanone	0.2
17	11.142	unknown	0.54
18	11.288	Linalool	1.09
19	11.827	Nona-1,3,7-triene <4,8-dimethyl-, (E)->	0.16
20	11.992	trans-2-Pinanol	0.14
21	12.785	Camphore	0.18
22	13.102	Citronellal	0.34
23	13.559	Borneol	1.49
24	13.924	Terpinen-4-ol	0.29
25	14.132	Isogeranial	0.3

26	14.412	Cryptone	0.76
27	14.592	Myrtenal	0.17
28	15.797	Citronellol	1.13
29	16.267	Neral	6.11
30	16.694	Geraniol	1.41
31	16.821	(2E)-decenal	0.29
32	17.329	Geranial	8.21
33	17.679	Bornyl acetate	0.52
34	17.902	2-Undecanone	0.43
35	19.841	Citronellol acetate	0.18
36	20.213	Cyclosativene	0.16
37	20.563	Copaene	0.36
38	20.835	Geranyl acetate	0.5
39	21.086	β-Elemene	0.25
40	21.513	α-Fenebrene	0.2
41	21.952	(+)(E)-Caryophyllene	0.17
42	22.876	9-epi-Caryophyllene	0.15
43	23.109	trans-β-bergamotene	0.58
44	23.247	9-epi-Caryophyllene	0.31
45	23.699	α-Selinene	0.51
46	23.909	β-Cubebene	1.71
47	23.992	unknown	3.79
48	24.501	α-Funebrene	12.51
49	24.602	Cadina-3,5-diene	0.78
50	24.671	unknown	0.4
51	24.813	α-Farnesene	6.51
52	24.899	cis-Muurola-4(14),5-diene	0.63
53	25	unknown	0.56
54	25.074	(-)-α-Panasinsen	0.54
55	25.307	Sesquisabinene	6.47
56	25.451	(E)-γ-Bisabolene	0.97
57	25.658	1.α.,10.α.-Epoxy-amorph-4-ene	0.43
58	25.758	Isoaromadendrene epoxide	0.39
59	25.994	Elemol	0.41
60	26.05	7-epi-trans-Sesquisabinene hydrate	0.48
61	26.197	Germacrene B	0.72
62	26.334	E-Nerolidol	1.47
63	26.705	Germacrene D-4-ol	0.25
64	26.809	Cadin-4-en-10-ol	0.36
65	26.937	Caryophyllene oxide	0.2
66	27.107	7-epi-trans-Sesquisabinene hydrate	0.64
67	27.327	Humulane-1,6-dien-3-ol	0.17
68	27.567	α-Eudesmol	0.18
69	27.788	α-(−)-bisabolol	0.97
70	27.992	Epi-Eudesmol	0.56
71	28.19	Ledene oxide-(I)	0.36
72	28.266	α-bisabolol	0.66
73	28.6	Copaborneol	0.37
74	28.836	β-Eudesmol	0.44
75	28.941	Cadin-4-en-10-ol	0.32
76	29.063	unknown	0.28
77	29.347	α-Elemol	0.27

78	29.461	Valeranone	0.39
79	29.875	α-bisabolol	0.59
80	29.948	Carotol	0.23
81	30.06	Dehydronerolidol	0.34
82	30.537	Cholestan-3-ol, 5,6-epoxy-, (3.beta.,5.alpha.,6.alpha.)-	0.24
83	30.577	Farnesal<(E,E)->	0.2
84	30.748	Farnesol	0.14
85	30.913	Benzene, 1-(3-cyclopentylpropyl)-2,4-dimethyl-	0.17
86	31.3	Farnesal<(E,E)->	0.23
87	31.527	α-trans-Bergamotenol	0.24
88	33.007	Bicyclo[10.1.0]trideca-4,8-diene<13-oxa-,trimethyl->	0.15
Total area			**100**

Table 4c: Chemical composition of ginger oil from Gorkha analyzed by GC-MS.
R. time: Retention time

Antibacterial properties

The zone of inhibition shown by EE and EEROE from Sindhupalchowk, Tanahu and Gorkha are comparable to that of Chloramphenicol antibiotic (Table 5). The zone of inhibition of each microbial organism is also comparable with EE and EEROE. Most of the studies suggested that 6-10 % ethanolic extract of ginger rhizome possessed the antimicrobial activities against different pathogenic microorganisms [18,21,22].

Ginger	Crude extract	*Staphylococus aureus*			*Escherichia coli*			*Bacillus substillus*			*Klebsella spp.*		
Sindhu	EE	15.0	±	1.4	15.5	±	0.7	9.5	±	0.7	14.0	±	3.1
	EEROE	18.3	±	1.8	17.5	±	0.7	14.7	±	1.8	16.3	±	1.5
Tanahu	EE	17.7	±	1.1	19.7	±	1.1	16.5	±	3.5	15.0	±	0.0
	EEROE	12.3	±	1.1	21.3	±	1.5	16.3	±	1.1	14.0	±	0.7
Gorkha	EE	14.0	±	0.0	15.5	±	2.1	10.7	±	1.1	11.7	±	1.1
	EEROE	12.0	±	0.0	19.5	±	0.7	18.0	±	2.5	15.0	±	0.7
chloramphenicol		15.0	±	1.2	17.0	±	1.4	14.0	±	0.7	20.6	±	0.4

Table 5: Antimicrobial screening of 8% Ethanolic extract of ginger from Sindhupalchok, Tanahu and Gorkha.
Data are expressed as mean ±SEM (n=3).
EE: Ethanolic extract, EEROE: Ethanolic extract of residue after oil extraction
Essential oil did not possess the antimicrobial activity on these bacteria

Antioxidant activities

Essential oils, EE and EEROE from Sindhupalchowk, Tanahu and Gorkha were evaluated for their free radical scavenging activity. All the extracts from three regions showed the similar antioxidant activities as compared to that of ascorbic acid. These findings suggest that the ginger extracts could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. Polyphenolics are the major plant compounds with antioxidant activity and it is likely that the activity of the extract is due to their redox properties [23] (Figure 1).

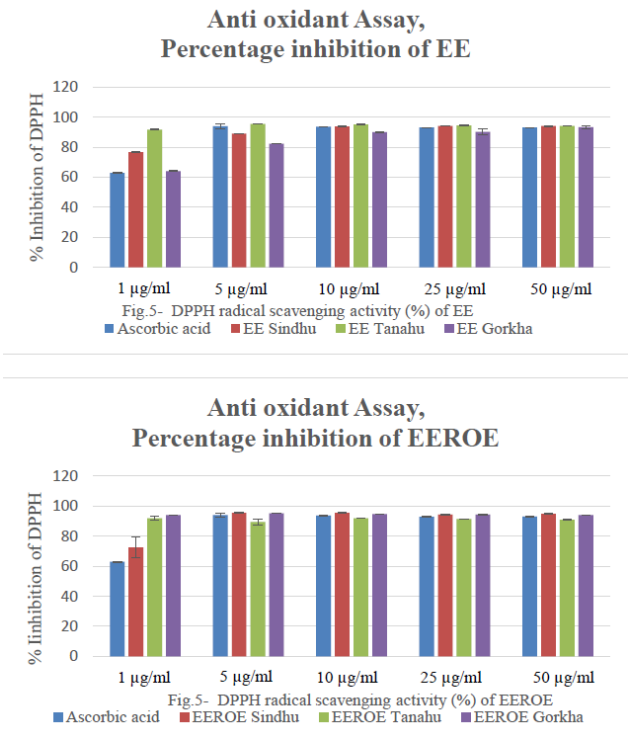

Figure 1: Antioxidant Assay of ethanolic extract of ginger of Sindhupalchok, Tanahu and Gorkha.
EE: Ethanolic extract, EEROE: Ethanolic extract of residue after oil extraction. Essential oil did not possess the antioxidant activity.
Data are expressed as mean ± SEM (n=3).

Moreover, the ginger oils of Sindhupalchowk, Tanahu and Gorkha showed no antioxidant activity (Figure 1) which is similar to the findings of Roses et al., 2008 [24]. But, it had been reported that ginger essential oils exhibited very excellent free radical scavenging activity at very low concentration [25]. This difference in antioxidant activity might be due to the type of cultivation and variations in plant type and growth, climate, season, temperature and soil conditions.

Conclusion

The three ginger samples showed some deviation and similarities in their volatile oil composition. The results also showed that the ginger ethanolic extracts exhibited significant antioxidant and antimicrobial activities. Since, they have exhibited moderate to significant antimicrobial properties, they can be used in the treatment of many bacterial and fungal diseases as well as a naturally food additives and preservatives which considered in new applications of food technology.

Authors' contributions

K.B had supervised the project, carried out the experimental plan and prepares the manuscript. B.P and S.M had equally contributed in the implementation of the experimental section, summarised and discussed the experimental results. S.A had advised on all aspects of the project and writing the manuscript. All authors read and approved the final manuscript.

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