Indian Journal of Natural Products and Resources
Vol. 14(4), December 2023, pp. 591-601
DOI: 10.56042/ijnpr.v14i4.5889

The essential oil composition, antimicrobial activity and antioxidant assay of the extracts from aerial parts of *Dicliptera roxburghiana* Nees.

Rajendra Prasad[1], Anand B. Melkani[1]*, Lalit S. Bisht[1], Lalit Mohan[1], Archana N. Sah[2] and Shubhangi Mehra[2]

[1]Department of Chemistry, Kumaun University, Nainital 263002, Uttarakhand, India

[2]Department of Pharmaceutical Sciences, Kumaun University, Bhimtal 263136, Uttarakhand, India

Received 13 September 2022; revised received 22 September2023; accepted 20 October 2023

Dicliptera roxburghiana Nees. is a shrub belonging to the family *Acanthaceae*. The present study was undertaken because of the medicinal importance of this genus and the need for a report on its essential oil composition and antibacterial activity. Essential oil from the whole aerial part, including flowers, of the plant was extracted by steam distillation method and analysed by GC-FID and GC-MS. In all, sixty-nine compounds constituting 85.5% of the total oil composition have been identified. The essential oil contained high concentrations of non-terpenic compounds (33.5%), while the terpenoid compounds had a higher concentration still (52.0%). The unidentified portion contained several minor constituents with ambiguous Mass Spectra. The major constituents of the essential oil were identified as *β*-panasinsene (11.2%), phytol (10.0%), pentadecanal (8.6%), *cis, cis, cis*-7,10,13-hexadecatrienal (7.6%), linalool (4.2%) and cameroonan -7-α-ol (4.2%). The antioxidant activity of four different extracts (whole aerial parts)was performed by two methods, namely, DPPH assay and metal chelating assay. Significant results were obtained in each case at 100 µg/mL concentration. For antibacterial screening, the essential oil exhibited the highest activity against Gram-negative *Pasteurella multocida* (15.66±0.57 mm) and Gram-positive *Bacillus subtilis* (13.00±1.00 mm). The highest activity of ethyl acetate extract was recorded against Gram-negative *Pasteurella multocida* (12.00±0.00 mm). The hexane extract of the plant was found to be most active against Gram-positive *Enterococcus faecalis* (13.33±0.57 mm). The chloroform extract showed the highest activity against Gram-negative *Xanthomonas phaseoli* (16.33±1.15 mm) and Gram-positive *Enterococcus faecalis* (12.00±1.00 mm). For methanol extract, the highest activity was observed against Gram-negative *Agrobacterium tumefaciens* (10.66±0.57 mm) and Gram-positive *Enterococcus faecalis* (10.33±0.57 mm). The essential oil exhibited the highest antifungal activity against the fungal strains, namely, *Aspergillus niger* (17.66±0.57 mm), followed by *Aspergillus flavus* (16.66±0.57 mm) and *Candida albicans* (16.33±0.57 mm). The essential oil and extracts from this plant species may be utilised to control various microbial infectious diseases and serve as an antioxidant supplement.

Keywords: Antibacterial, Antifungal, Antioxidant assay, *Dicliptera roxburghiana* Nees., Essential oil composition

IPC code; Int. cl. (2021.01)– A61K 36/00, A61P 31/00, A61P 39/00

Introduction

Dicliptera roxburghiana is a common shrub found to grow wild in the Uttarakhand Himalayan region and is locally known as *Pataru*. There are 150 species of genus *Dicliptera* distributed in warm and tropical areas of the world[1]. In India, 21 species and nine varieties of genus *Dicliptera* have been reported[2], of which one species and one of its varieties are growing wild in Kumaun and Garhwal regions of Uttarakhand, i.e. *D. roxburghiana* Nees. and *D. roxburghiana* var. *Bupleuroides*[3].

The genus *Dicliptera* is known for its various medicinal uses, e.g., the leaf infusion of *D. laxata* has application as a poison antidote and is locally used in Southern Uganda[4]. Similarly, *D. verticillata* is reported to have traditional use to cure diarrhoea and malaria in Burkina Faso. The leaves of the species are used in making various soups in India[5]. The plant species also find applications in flavouring and spicing[6]. It is further reported that the ethanol extract of *D. verticillate* was analysed for its antioxidant assay and antimicrobial assay (broth macro-dilution method). The results showed moderate antioxidant activity (63.9%, IC_{50} 40.16 µg/mL) and bactericidal effects against *S. aureus, K. pneumoniae and E. coli*[7]. *Dicliptera ghaticas Santapau* is another important species of this genus that is reported to possess significant antioxidant activity[8]. *β*-Sitosterol and vanillic acid isolated from *D. bupleuroides* exhibit

*Correspondent author
Email: melkanianand@gmail.com

significant antibacterial activity with MIC values between 0.467 and 0.809 mg/mL[9].

D. roxburghiana is known for its ethnomedicinal uses; e.g., its powder is reported for manufacturing common tonic[10], which is considered to help heal wounds[11] and is non-toxic in nature[12]. According to a previous report, flavonoids *viz*; apigenin, luteolin, kaempferol and apigenin-7-O-glucoside and saturated fatty acids (C-15 to C-31) have been extracted and identified from *D. roxburghiana*[13]. Also, carotenoids, flavonoids, α-amino acids, daucosterol, betulin, and long-chain aliphatic hydrocarbons have been separated from *D. chinensis* and *D. roxburghiana*[14].

The present study aimed to investigate the composition of essential oil from whole aerial parts of *D. roxburghiana* and to assess its antimicrobial potential. Although, several antimicrobial drugs are in use and prepared previously, yet the scientific community is continuously searching for various other safer chemicals and highly potent antimicrobial drugs to treat various severe microbial infections with nearly 100% efficacy. In this study, ten bacterial and three fungal strains are taken since these are causative agents for several diseases in humans, animals and plants. For example, *Staphylococcus aureus* causes skin infections, pneumonia, endocarditis, osteomyelitis and others problems[15], similarly, *Klebsiella pneumonia* causes pneumonia, bloodstream infections, UTIs and other severe infections in body[16]. *Escherichia coli* is responsible for UTIs, abdominal and pelvic infections, pneumonia, bacteremia and other problems[17], likewise, *Bacillus subtilis* causes serious infectious complications, central nervous system infection, meningitis, pneumonia, septicemia etc[18]. *Salmonella enterica enterica* is a causative agent for typhoid and paratyphoid fever[19], similarly, *Enterococcus faecalis* causes UTIs, bacteremia, meningitis, intra-abdominal infections etc[20]. *Pasteurella multocida* causes respiratory disease and other related problems[21], similarly, *Agrobacterium tumefaciens causes* crown gall disease (formation of tumour)[22]. *Erwinia chrysanthemi* causes bacterial stem and root rot problems[23] and *Xanthomonas phaseoli* is responsible for common bacterial blight of bean (CBB) (an uncontrolled disease worldwide)[24]. To develop safer and sustainable drugs against these pathogens plant-based medicines may play crucial role.

The essential oil from *D. roxburghiana* was not explored previously regarding its efficacy against microorganisms and to find out its chemical composition. So, the present study is aimed to find out the possibility, whether the essential oil and extracts of this plant may be a source of such chemicals or not. Also, it seeks to examine the antioxidant and antimicrobial activities of the extracts prepared using hexane, chloroform, ethyl acetate and methanol. Although there are some reports on antioxidant activity from the extracts of this plant, our results are quite different from the previous reports[25], since the plant material collected for the present study was from geographically different locations.

Materials and Methods

Plant material

The plant material of *Dicliptera roxburghiana* Nees. was collected from Nainital (Uttarakhand), India, from an altitude of 2100 m during its flowering stage. The whole aerial parts of the plant were used to extract the essential oil. The plant specimen was identified from the Botany Department, Kumaun University, Nainital and its botanical identity was further confirmed by the Botanical Survey of India, Dehradun. The herbarium specimen was submitted to the BSI, Dehradun, for future reference (Herbarium Acc. No. BSI/NC 112186).

Extraction of essential oil

The aerial parts of *Dicliptera roxburghiana* Nees. (5 kg) were subjected to steam distillation using a copper still fitted with spiral glass condensers. The distillates obtained were saturated with sodium chloride; 30 mL of *n*-hexane per 2.0 L of aqueous distillate was added to the mixture, and it was shaken thoroughly several times. The oil layer comes with *n*-hexane and was separated with the help of a separatory funnel. The process was repeated with the second installment of 30 mL hexane. Anhydrous sodium sulfate was added to the hexane extract to remove any aqueous content. Finally, the solvent was removed using a Rotary evaporator at reduced pressure and 30°C to obtain the pure essential oil.

GC-FID and GC-MS analysis

GC analysis of the essential oil was done by using a Shimadzu GC-2010 plus gas chromatograph system attached with an FID detector and a capillary column Rtx-5MS (30 m x 0.25 mm) with a film thickness of 0.25 µm. The carrier gas used was Nitrogen. The oven temperature was initially programmed at 50°C for the first 10 minutes, then programmed at 3°C/min from 50 to 240°C and finally kept isothermal at 240°C for

the last 5 minutes with a total elapsed time of 78.3 minutes. The percentage composition data were taken from the area percentage data by rounding off to the first place of the decimal.To determine the Retention Indices of compounds, a mixture of essential oil and a standard sample of *n*-alkanes (C_8-C_{33}) was injected and analysed under identical GC conditions.

A Shimadzu MS-QP-2010 Plus system interfaced with a GC-2010 gas chromatograph, and an Rtx-5 MS capillary column (30 m × 0.25 mm) with a film thickness of 0.25µm was used for the GC-MS analysis of the oil sample. Helium was used as the carrier gas. The mass spectral data were taken under the EI condition at 70eV. The oven temperature was set at 50°C for the first 10 minutes, then increased at a rate of 3°C/min from 50 to 230°C and set isothermal at 230°C for the last 10 minutes.

Preparation of different solvent extracts

After collection from the field, the plant material of *D. roxburghiana* Nees. (8 kg) was shade-dried. The dried plant material was ground to powdered form, poured into a percolator and a sufficient quantity of methanol was added to it. The dipped material was regularly shaken thoroughly several times daily and kept for three days. On the fourth day, the extract was separated from the crude material by filtration while the methanol was removed using a Rotary evaporator. The extract obtained was mixed with coarse silica, and serial solvent extraction was performed using *n*-hexane, chloroform, ethyl acetate and methanol, respectively. The extraction was done using the cold extraction method. Initially, the crude extract thoroughly mixed with coarse silica was dipped in n-hexane and kept overnight. The mixture was thoroughly shaken several times and filtered off. After removing hexane from the filtrate, n-hexane extract was obtained. The residue left behind was then treated with chloroform, and similarly, chloroform extract was obtained.

Similarly, ethyl acetate extract was obtained from the residue left in the chloroform step, and methanol extract was obtained from the residue left in the ethyl acetate step. The solvent from each extract was removed with the help of a Rotary evaporator at reduced pressure. In this way, four different extracts, namely, *n*-hexane, chloroform, ethyl acetate and methanol extract, were obtained.

Antioxidant assay

DPPH radical scavenging activity

The free radical scavenging activity of extracts was measured by the DPPH (1,1-diphenyl-2-picryl hydrazyl) method[26]. Various concentrations (10, 20, 40, 60, 80, 100 µg/mL) of plant extract (sample) and ascorbic acid (standard) were prepared in 70% ethanol in different test tubes. After this, the methanol solution of DPPH (0.1mM or 100µM) was prepared, and 1 mL was added to every test tube, shaken rapidly and allowed to stand for 30 minutes at 27ºC. As described above, a control was prepared without a standard or sample. A mixture of 3 mL 70% ethanol and 1 mL methanol was used for the baseline correction. Absorbance was measured at 517 nm. All the tests were performed in triplicate, and the results were averaged out. Radical scavenging activity was expressed as the inhibition percentage and was calculated by the formula:

$$\%\ \text{Radical scavenging activity} = \frac{A_{Control} - A_{Sample}}{A_{Control}} X\ 100$$

Where $A_{Control}$ is the absorbance of the control, and A_{Sample} is the absorbance of the test sample

Metal chelating activity

The chelating effect was determined using standard method[27]. Various concentrations (10, 20, 40, 60, 80, 100 µg/mL) of the plant extract were prepared in 70% ethanol. Then, 1mL of the extract was added to 2 mM ferric chloride (0.1 mL). The reaction was initiated by adding 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left for 10 minutes at room temperature. The measurement of absorbance for solution was done at 562 nm. Ascorbic acid was used as a positive control. All the tests were run in triplicate and averaged out. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated by the given formula:

$$\text{Metal chelating activity} = \frac{A_{Control} - A_{Sample}}{A_{Control}} X\ 100$$

Where $A_{Control}$ is the absorbance for the control reaction (without plant extract), and A_{Sample} is the absorbance in the presence of a plant extract.

***In-vitro* antimicrobial activity**

Microbial culture

For antibacterial activity, seven animal pathogens viz; *Escherichia coli* (MTCC 443), *Bacillus subtilis*

(MTCC 121), *Salmonella enteric enterica* (MTCC 3223), *Enterococcus faecalis* (MTCC 439), *Klebsiella pneumonia* (MTCC 109), *Pasteurella multocida* (MTCC 1148), *Staphylococcus aureus* (MTCC 737) and three plant pathogens e.g., *Agrobacterium tumefaciens* (MTCC 609), *Erwinia chrysanthemi* (KUMSCC 328), *Xanthomonas phaseoli* (KUMSCC 327) were taken. For antifungal assay, we used three fungal strains named *Aspergillus niger* (MTCC 1344), *Candida albicans* (MTCC 227) and *Aspergillus flavus* (MTCC 277).

Antibacterial activity

The standard method was used to assess the antibacterial activity of essential oils and solvent extracts[28,29]. The initial screening of the oil samples was conducted at 1000 µL/mL concentration. Erythromycin (15 µg/disc, Hi-media) and Streptomycin (30 µg/disc, Hi-media)were used as positive control, whereas hexane was used as negative control against all the bacterial strains, and the inhibition zone was compared against the essential oil disc. Minimum Inhibitory Concentration (MIC) was observed at five different concentrations of the oil, i.e. 500, 250, 125, 62.5 and 31.25 µL/mL. The MIC was obtained only for those pathogens against which the oil showed inhibition ≥10 mm in the initial screening of essential oil. All the experiments were done in triplicate. Nutrient agar media of pH=7.3±0.2 was used and autoclaved at 121°C for 30 min and a pressure of 15 psi. The streaking of pathogens on agar plates was done in radial pattern[19], rotating it about 60° every time to confirm the even inoculums of pathogens. After pathogens inoculation on Petri plates was done, two Whatman no. 1 filter paper discs of 5mm diameter were applied on the plates, with one dipped in the oil sample and another one dipped in n-hexane (negative control). In comparison, two discs of standard antibiotics (positive control) were applied on each petri plate with the help of forceps. Petri plates were placed in the incubator at (37±1°C). The plates were examined after 24 hours of incubation for growth and inhibition. The zone of inhibition was taken in diameter and measured to the nearest millimeter (mm).

Minimum Bactericidal Concentration (MBC) was evaluated by taking a small fraction of media from a clearly inhibited zone of MIC-tested petri plates onto the fresh Petri plates containing fresh nutrient agar media. The petri plates were then incubated at 37±1°C. The concentrations with no growth of bacterial strains were recorded as MBC. The above procedure for the antibacterial assay was published in our previous publications[30-32].

Antifungal activity

Disc diffusion method[28] was applied to assess the antifungal activity of essential oil. The Potato Dextrose Agar media was sterilized in an autoclave at 121°C for 30 minutes and at 15 psi pressure. After sterilization and lowering the temperature to 50°C, 20 mL media was poured into sterilized petri plates (80 mm id) in a laminar flow. After solidification of media on Petri plates, one Whatman no.1 filter paper disc (5 mm diameter) was dipped in essential oil (1000 µL/mL concentration). Another disc of standard antibiotics of Clotrimazole (10 µg/disc, as a positive control) was applied on the prepared petri plates with the help of forceps at an equal distance from the edges of the Petri plates and opposite to each other. Forceps were sterilized after every application by dipping them in spirit and then inflaming. The seven-day-old fungal culture was applied at the centre of the Petri plate with the help of a sterilized nichrome loop by dipping its tip in the fungal strain. Every set of experiments was done in triplicate. The Petri plates were then tightly sealed, wrapped by parafilm tape and placed in an incubation chamber for seven days at 27±1°C. Examination of Petri plates was done after 7 days of incubation by measuring the zone of inhibition in diameter to the nearest millimeter with the help of a ruler. The MIC was assessed at four different concentrations of oil samples, i.e. at 500, 250, 125, and 62.5 µL/mL. The concentrations were prepared through the serial dilution method. The preparation of Petri plates and application of sample was done as in case of antibacterial activity, except the four concentrations of oil samples were applied clockwise, and fungal strains were applied at the centre of Petri plates.

Statistical analysis

The data obtained from triplicate independent experiment as mean±standard deviation. One-way ANOVA and Duncan test were performed for statistical differences among the samples by using SPSS16.0 at a level of significance α=0.05.

Results

Chemical composition of the essential oil

The essential oil from *D. roxburghiana* was observed with a characteristic aromatic odour and

yellowish colour. The oil yield of the essential oil was very low and found to be 0.01% w/w based on the fresh weight of the plant. Several different chemical constituents have been identified from the essential oil. The GC of the oil sample showed more than two hundred peaks. However, only sixty-nine constituents with 85.5% of the total oil composition have been identified (Fig. 1). The essential oil contained a high concentration of non-terpenic compounds (33.5%). The terpenoid constituents of the essential oil were found to be 52.0%. The identification of the compounds was achieved by calculating retention indices for the compounds and then comparing their Retention Indices and mass spectra with those reported in the literature[33] and from the inbuilt library.

The major compounds of the essential oil were identified as *β*-panasinsene (11.2%), phytol (10.0%), pentadecanal (8.6%), *cis, cis, cis*-7,10,13-hexadecatrienal (7.6%), linalool (4.2%), cameroonan-7-*α*-ol (4.2%) (Table 1). The literature search did not reveal report/s on essential oil composition from any

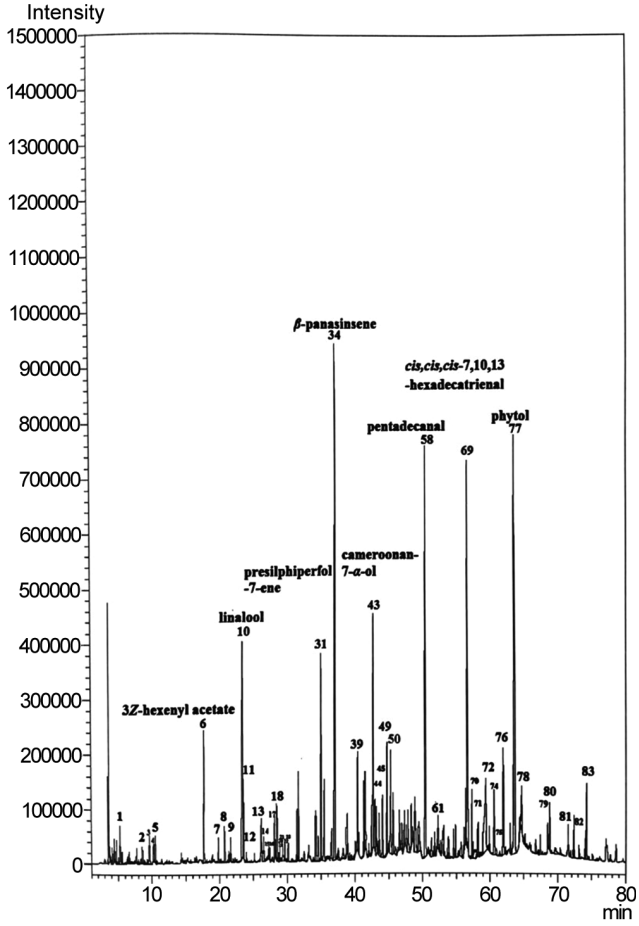

Fig. 1 — Gas Chromatogram of the essential oil of *D. roxburghiana* Nees.

Table 1 — The composition of essential oil from whole aerial parts of *Dicliptera roxburghiana* Nees.

S. No.	Compound[A]	$RI_{observed}$	$RI_{reported}$	Percentage
1	*n*-octane	800	800	0.4
2	2*E*-hexenal	856	855	0.1
3	*n*-nonane	894	900	0.3
4	*n*-heptanal	900	902	0.2
5	1-ethyl-4-methyl cyclohexane	933	941	0.3
6	3*Z*-hexenyl acetate	1012	1005	2.0
7	phenyl acetaldehyde	1049	1042	0.3
8	2*E*-octen-1-al	1063	1054	0.4
9	*n*-octanol	1077	1068	0.3
10	Linalool	1104	1096	4.2
11	*n*-nonanal	1107	1100	1.0
12	2,2-dimethyl-3,4-octadienal	1116	1113	0.1
13	2*E*,6*Z*-nonadienal	1158	1154	0.5
14	3*E*,6*Z*-nonadienol	1164	1153	0.4
15	umbellulone	1179	1171	0.1
16	*α*-terpineol	1186	1188	0.1
17	methyl salicylate	1193	1191	0.5
18	Safranal	1196	1196	0.6
19	Dodecane	1200	1200	0.1
20	methyl-*α*-cyclogeranate	1207	1197	0.2
21	2*E*,4*E*-nonadienal	1223	1212	0.2
22	*β*-cyclocitral	1229	1219	0.2
23	Geraniol	1260	1252	1.0
24	dec-(2*E*)-enal	1266	1263	1.2
25	perilla aldehyde	1280	1271	0.2
26	3*E*,6*Z*- nonadienol acetate	1296	1297	0.1
27	*p*-vinyl-guaiacol	1320	1309	1.0
28	presilphiperfol-7-ene	1337	1336	3.0
29	*δ*-elemene	1342	1338	1.2
30	longicyclene	1372	1374	0.4
31	*β*-panasinsene	1382	1382	11.2
32	(*E*)-*β*-damascenone	1388	1384	0.1
33	*β*-caryophyllene	1424	1419	0.6
34	sesquisabinene	1449	1459	0.3
35	*allo*- aromadendrene	1456	1460	0.1
36	(*E*)-*β*-farnesene	1461	1456	1.1
37	*β*-acoradiene	1479	1470	0.2
38	*γ*-curcumene	1487	1482	1.2
39	(*E*)-*β*-ionone	1492	1488	0.2
40	cameroonan-7-*α*-ol	1517	1511	4.2
41	silphiperfolan-7-*β*-ol	1524	1520	1.0
42	*δ*-cadinene	1530	1523	1.0
43	nopsan-4-ol	1535	1531	0.2
44	italicene ether	1541	1537	1.0
45	hedycaryol	1552	1548	1.0
46	prenopsan-8-ol	1582	1577	1.6

(*Contd.*)

Table 1 — The composition of essential oil from whole aerial parts of *Dicliptera roxburghiana* Nees. (*Contd.*)

S. No.	Compound[A]	$RI_{observed}$	$RI_{reported}$	Percentage
47	(*E*)-dihydro-apofarnesol	1591	1591	1.3
48	2, (7*Z*)-bisaboladien-4-ol	1627	1619	0.4
49	*α*-acorenol	1638	1633	0.6
50	gossonorol	1643	1636	0.4
51	*epi*-*α*-muurolol	1651	1642	1.0
52	*α*-cadinol	1664	1654	1.0
53	*α*-bisabolol	1691	1685	0.5
54	germacra-4(15),5,10(14)-trien-1-*α*-ol	1695	1686	0.6
55	pentadecanal	1710	1701	8.6
56	6*S*,7*R*-bisabolon	1755	1749	0.2
57	xanthorrhizol	1760	1753	0.3
58	tetradecanoic acid	1774	1769	0.7
59	1-octadecene	1783	1790	0.2
60	hexadecanal	1810	1800	0.2
61	cyclopentadecanolide	1839	1833	0.4
62	*n*-hexadecanol	1887	1875	0.4
63	*cis,cis,cis*-7,10,13-hexadecatrienal	1904	- -	7.6
64	musk ambrette	1931	1930	1.0
65	hexadecanoic acid	2051	1968	3.0
66	*n*-heneicosane	2101	2100	0.5
67	Phytol	2244	- -	10.0
68	*n*-pentacosane	2502	2500	0.8
69	*n*-hexacosane	2597	2600	0.2

A: Compounds listed in the elution order; $RI_{Observed}$: Calculated retention indices; relative to the *n*- alkanes (C_7–C_{34}) on Rtx-5 MS capillary column as described in the Experimental section; $RI_{Reported}$: Reported Retention indices in the literature[23]

Dicliptera species hence a comparison on essential oil composition and chemotaxonomic relation could not be accomplished.

Antioxidant activity

DPPH Radical scavenging activity

Four different solvent extracts of *D. roxburghiana* were assessed for their DPPH radical scavenging potential, taking ascorbic acid as standard. DPPH is a very stable free radical with an absorbance maximum of 517 nm. It can be easily scavenged in the presence of an antioxidant and converted into 1,1-diphenyl-2-picrylhydrazine. The intensity of colouration indicates the scavenging potency of the extract with antioxidant properties[34]. The lower the absorbance of the reaction mixture, the greater its free radical scavenging potency. The free radical scavenging potency gradually increases with the increase in the concentration of the reaction mixture.

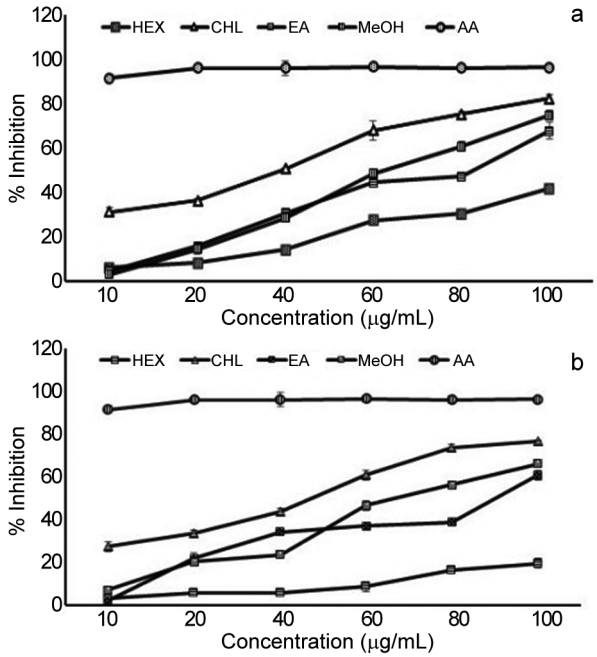

Fig. 2 — Percentage inhibition of extracts of *D. roxburghiana* and standard at different concentrations (AA= ascorbic acid, HEX=hexane extract, CHL= chloroform extract, EA= ethyl acetate extract, MeOH= methanol extract). a) DPPH assay; and b) Metal chelating antioxidant assay. Data observed are mean ± SD (n=3, $P<0.05$).

Nearly all four solvent extracts were observed with a significant radical scavenging potential compared to the standard antioxidant. The highest DPPH radical scavenging potential of *D. roxburghiana* was observed at 100 µg/mL concentration of the sample and follows the order: chloroform extract (82.2%), methanol extract (74.7%), ethyl acetate (67.7%) and hexane extract (41.8%). The activity of four different extracts was slightly lower than the standard ascorbic acid (96.1%), but chloroform, methanol and ethyl acetate extracts showed quite significant values. The absorbance values at different concentrations of sample and standard are shown in Fig. 2a. A previous report on DPPH assay of different extracts of *D. roxburghiana* (from Pakistan) showed nearly similar results in the order of chloroform extract> Methanol extract> Ethyl acetate extract> n-hexane extract[25].

The literature report on the DPPH radical scavenging assay from *D. verticillate* indicated that the crude ethanolic extract of the plant showed the best inhibition percentage (PI: 63.90% at 125 mg/mL)[7]. In another report on DPPH antioxidant assay, the isolated compounds of *D. bupleuroides* showed significant activity. Compound *β*- sitosterol with an IC_{50} value of 198.87 µg/mL and vanillic acid

with an IC_{50} value equal to 92.68 µg/mL were observed with potent antioxidant power[9]. Our results are somewhat comparable to these results. The results of the present investigation indicated that these extracts might be utilised as free radical scavengers and might serve as potential antioxidants.

Metal chelating activity

Iron is a reactive metal and may catalyse the oxidative changes in protein, lipids, and other components of the cell. Also, the oxidative damage and liposome peroxidation in proteins is accelerated by a Fenton reaction where Fe^{2+} ions catalyse the conversion of H_2O_2 to the hydroxyl radical with the production of ferric ion[34].

Ferrozine is capable of forming complex compounds with Fe^{2+}. The complex formation is disrupted in the presence of chelating substances, resulting in a decrease in the red colour of the complex. By measuring the colour reduction, one can estimate the chelating activity of a chelator[35,36]. The chelating agents can form σ-bonds with metal and are effective as secondary antioxidants because they can reduce the redox potential and stabilise the oxidised form of metal ion[37].

The lower absorbance of the reaction mixture indicates higher chelating activity. By comparing our results of metal chelating activity of different solvent extracts of *D. roxburghiana,* it was observed that at 100 µg/mL concentration of the sample, the metal-chelating action of different extracts follows the order: chloroform extract (76.4%), ethanol extract (66.2%), ethyl acetate extract (60.8%) and hexane extract (19.7%). These results indicated that the chloroform, methanol and ethyl acetate extracts of *D. roxburghiana* might serve as a good chelator. The absorbance tested at different concentrations for extracts and standards is given in Fig. 2b.

Antibacterial activity

The essential oil from aerial parts of *D. roxburghiana* displayed the highest inhibitory action against Gram-negative pathogens *P. multocida*, *X. phaseoli*, *K. Pneumonia* and *S. enterica*, while towards Gram-positive pathogens, the oil displayed the highest zone of inhibition against *B. subtilis*. The oil showed slightly lower activity against other pathogens taken for the study (Table 2). The minimum inhibitory concentration (MIC) for essential oil was observed at 31.25 µL/mL against *K. pneumoniae, P. multocida, E. faecalis*, *A. tumefaciens*, *X. phaseoli, B. subtilis, S. aureus, E. chrysanthemi* and *S. enterica.* Minimum bactericidal concentration (MBC) was observed at 500 µL/mL concentration against *K. pneumoniae, P. multocida, E. faecalis*, *B. subtilis*, *S. aureus* and *S. enterica,* while it was at 250 µL/mL against *X. phaseoli* (Table 3).

Table 2 — Antibacterial screening of essential oil from *D. roxburghiana* Nees. (1000 µL/mL)

Microorganism (Bacteria)	Zone of Inhibition (mm)		
	STRE	ERY	DRN (oil)
K. pneumoniae	22.00^{d}±1.00	25.00^{d}±1.00	14.00^{b}±1.00
E. coli	18.33^{c}±0.58	18.66^{c}±0.58	09.00^{a}±1.00
P. multocida	23.33^{d}±0.58	18.00^{c}±0.00	15.66^{c}±0.57
E. faecalis	22.00^{d}±0.00	13.66^{b}±1.15	10.33^{a}±0.57
A. tumefaciens	30.00^{f}±1.00	28.33^{e}±0.58	10.33^{a}±0.57
X. phaseoli	25.66^{e}±1.15	12.00^{b}±0.00	$14.33^{b, c}$±1.15
B. subtilis	25.00^{e}±1.00	12.33^{b}±0.58	13.00^{b}±1.00
S. aureus	18.00^{c}±1.00	10.33^{a}±0.58	10.33^{a}±0.57
E chrysanthemi	12.00^{a}±0.00	12.00^{b}±2.00	10.00^{a}±1.00
S. enterica enterica	16.00^{b}±0.00	12.66^{b}±0.58	13.33^{b}±0.57

DRN: *Dicliptera roxburghiana* Nainital; STRE: Streptomycin; ERY: Erythromycin; a, b, c, d, e, f, and g indicate groups of homogenous subsets which are based on One-way ANOVA tests and Duncan tests at α = 0.05 level of significance.

The Ethyl acetate extract exhibited maximum efficacy against Gram-negative pathogens, *P. multocida*, *X. phaseoli* and *K. pneumoniae.* In contrast, the extract was most active against Gram-positive pathogens in the order: *E. faecalis* > *B. subtilis* > *S.aureus*. Similarly, the hexane extract of the plant showed maximum efficacy against Gram-negative pathogens: *A. tumefaciens*, *X. phaseoli*, *K. pneumoniae* and *E. coli*, while significant activity was recorded against Gram-positive pathogens *E. faecalis*, *B. subtilis* and *S. aureus*. The chloroform extract of the plant showed significant efficacy against Gram-negative pathogens: *X. phaseoli*, *K. pneumoniae*, *E. coli* and *E. chrysanthemi,* while maximum activity was recorded against Gram-positive pathogens *E. faecalis* and *B. subtilis*. The methanol extract showed maximum activity against Gram-negative pathogens in the order *A. tumefaciens*, *K. pneumoniae* and *S. enterica*. In contrast, maximum activity was recorded against Gram-positive pathogen *E. faecalis*. The extracts were less active against the remaining pathogens under study (Table 4).

The ethyl acetate extract of the species showed MIC at the lowest concentration of 31.25 µg/mL against *X. phaseoli* and *P. multocida*. In comparison, it was observed at 62.5 µg/mL against *K. pneumoniae, E. faecalis* and *B. subtilis*. The MBC

was observed at 500 µg/mL for *X. phaseoli, P. multocida, K. pneumoniae, E. faecalis* and *B. subtilis*. Likewise, the hexane extract of the species exhibited MIC at the lowest concentration of 31.25 µg/mL against *E. faecalis*, and MBC was observed at 500 µg/mL concentration (Table 5). For chloroform extract of the plant, MIC was observed at 31.25 µg/mL against *K. pneumoniae, X. phaseoli* and *E. faecalis*, while it was recorded at 62.5 µg/mL against *E. chrysanthemi* and *E. coli*. The MBC was observed at 500 µg/mL for *X. phaseoli* and *E. faecalis*. The methanol extract of the plant showed MIC at the concentration of 62.5 µg/mL against *A. tumefaciens*, and it was observed at 125 µg/mL against *E. faecalis*. The MBC against *A. tumefaciens* was observed at 500 µg/mL (Table 6).

Table 3 — Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for *D. roxburghiana* essential oil

Bacterial pathogens	Concentration (µL/mL)				
	500	250	125	62.5	31.25
K. pneumoniae	$11.66^{c,d}$±0.57*	11.00^{c}±1.000	9.66^{c}±0.570	7.33^{c}±1.15	06.33^{b}±0.57▲
P. multocida	12.33^{d}±0.57*	11.33^{c}±0.57	10.33^{c}±0.57	08.66^{d}±0.57	06.66^{b}±0.57▲
E. faecalis	08.33^{b}±0.57*	08.00^{b}±0.00	07.00^{b}±1.00	06.00b±0.00	06.00^{b}±0.00▲
A. tumefaciens	08.33^{b}±0.57	07.66^{b}±0.57	06.66^{b}±1.15	06.00^{b}±0.00	06.00^{b}±0.00▲
X. phaseoli	11.00^{c}±1.00	10.33^{c}±0.57*	09.33^{c}±0.57	07.33^{c}±0.57	06.33^{b}±0.57▲
B. subtilis	11.00^{c}±1.00*	10.33^{c}±0.57	09.00^{c}±1.00	$08.00^{c,d}$±1.00	06.33^{b}±0.57▲
S. aureus	08.33^{b}±0.57*	07.33^{b}±0.57	06.33^{b}±0.57	06.00^{b}±0.00	06.00^{b}±0.00▲
E. chrysanthemi	08.00^{b}±0.00	07.33^{b}±0.57	06.33^{b}±0.57	06.00^{b}±0.00	06.00^{b}±0.00▲
S. enterica	11.00^{c}±1.00*	10.33^{c}±0.57	09.00^{c}±1.00	$07.66^{c,d}$±0.57	06.00^{b}±0.00▲

*: Minimum Bactericidal Concentration (MBC); ▲: Minimum Inhibitory Concentration (MIC). The values are obtained through mean inhibition zone (mm) ± Standard deviations;a,b,c,d,e,f represent the group of homogeneous subsets based upon One-Way ANOVA analysis and Duncan test at a level of significance α = 0.05.

Table 4 — Antibacterial screening of four solvent extracts from *D. roxburghiana* Nees. (1000 µg/mL)

Microorganism (Bacteria)	Zone of inhibition (mm)					
	STRE	ERY	EA extract	HEX extract	CHL extract	MeOH extract
K. pneumoniae	22.00^{d}±1.00	25.00^{d}±1.00	11.66^{b}±0.57	$08.33^{c,d}$±0.57	$11.66^{d,e}$±0.57	09.33^{c}±0.57
E. coli	18.33^{c}±0.58	18.66^{c}±0.58	09.33^{a}±0.57	$07.33^{a,b,c}$±0.57	$33^{d,e}$±0.57	08.66^{c}±0.57
P. multocida	23.33^{d}±0.58	18.00^{c}±0.00	12.00^{b}±0.00	$07.00^{a,b}$±0.00	$07.66^{a,b}$±0.57	06.33^{b}±0.57
E. faecalis	22.00^{d}±0.00	13.66^{b}±1.15	11.33^{b}±0.57	13.33^{f}±0.57	12.00^{e}±1.00	10.33^{d}±0.57
A. tumefaciens	30.00^{f}±1.00	28.33^{e}±0.58	09.66^{a}±0.57	09.66^{e}±1.15	$08.66^{b,c}$±0.57	10.66^{d}±0.57
X. phaseoli	25.66^{e}±1.15	12.00^{b}±0.00	12.00^{b}±1.00	$09.00^{d,e}$±0.00	16.33^{f}±1.15	07.00^{b}±0.00
B. subtilis	25.00^{e}±1.00	12.33^{b}±0.58	11.00^{b}±0.00	$07.66^{b,c}$±0.57	$08.33^{b,c}$±0.57	08.33^{c}±0.57
S. aureus	18.00^{c}±1.00	10.33^{a}±0.58	09.00^{a}±0.00	$07.66^{b,c}$±0.57	07.00^{a}±0.00	05.66^{a}±0.57
E. chrysanthemi	12.00^{a}±0.00	12.00^{b}±2.00	08.66^{a}±0.57	$07.00^{a,b}$±0.00	10.66^{d}±0.57	08.66^{c}±0.57
S. enterica	16.00^{b}±0.00	12.66^{b}±0.58	09.33^{a}±0.57	06.33^{a}±0.57	09.33^{c}±0.57	09.33^{c}±0.57

EA: Ethyl acetate, HEX: Hexane, CHL: Chloroform, MeOH: Methanol; a, b, c, d, e, and f indicate groups of homogenous subsets which are based on One-way ANOVA tests and Duncan tests at α = 0.05 level of significance

Table 5 — Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for *D. roxburghiana* extract

Bacterial pathogens	Ethyl acetate extract concentration (µg/mL)				
	500	250	125	62.5	31.25
K. pneumoniae	09.33^{a}±0.57*	08.33^{a}±0.57	06.66^{a}±0.57	06.00^{a}±0.00▲	NA
P. multocida	09.66^{a}±0.57*	08.33^{a}±0.57	07.33^{a}±0.57	06.33^{a}±0.57	06.00^{c}±0.00▲
E. faecalis	09.00^{a}±0.00*	07.33^{a}±0.57	06.33^{a}±0.57	05.66^{a}±0.57▲	NA
X. phaseoli	09.66^{a}±0.57*	08.33^{a}±0.57	06.66^{a}±0.57	06.00^{a}±0.00	05.33^{b}±0.57▲
B. subtilis	09.00^{a}±0.00*	07.66^{a}±0.57	07.00^{a}±0.00	05.66^{a}±0.57▲	NA
	Hexane extract concentration (µg/mL)				
E. faecalis	10.00^{b}±0.00*	08.66^{a}±0.57	07.00^{a}±1.00	06.33^{a}±0.57	05.33^{a}±0.57▲

*: Minimum Bactericidal Concentration (MBC); ▲: Minimum Inhibitory Concentration (MIC). The values are obtained through mean inhibition zone (mm) ± Standard deviations; a,b,c,d,e,f represent the group of homogeneous subsets based upon One-Way ANOVA analysis and Duncan test at a level of significance α = 0.05.

Table 6 — Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for *D. roxburghiana* extract

Bacterial pathogens	Chloroform extract concentration (µg/mL)				
	500	250	125	62.5	31.25
K. pneumonia	09.33^{a}±0.57	07.66^{a}±0.57	06.66^{a}±0.57	06.00^{a}±0.0	005.00^{b}±0.00▲
E. coli	09.00^{a}±0.00	07.66^{a}±0.57	07.00^{a}±0.00	05.66^{a}±0.57▲	NA
E. faecalis	09.66^{a}±0.57*	08.33^{a}±0.57	06.66^{a}±0.57	06.00^{a}±0.00	05.33^{b}±0.57▲
X. phaseoli	12.33^{b}±0.57*	10.33^{b}±0.57	09.00^{b}±0.00	07.66^{b}±0.57	06.00^{c}±0.00▲
E. chrysanthemi	08.66^{a}±0.57	07.33^{a}±0.57	06.33^{a}±0.57	05.33^{a}±0.57▲	NA
	Methanol extract concentration (µg/mL)				
E. faecalis	08.33^{a}±0.57	07.00^{a}±0.00	06.00^{a}±0.00▲	NA	NA
A. tumefaciens	08.66^{a}±0.57*	08.00^{a}±0.00	06.33^{a}±0.57	05.33^{a}±0.57▲	NA

*: Minimum Bactericidal Concentration (MBC); ▲: Minimum Inhibitory Concentration (MIC). The values are obtained through mean inhibition zone (mm) ± Standard deviations; NA: Not active; a,b,c,d,e,f represents the group of homogeneous subsets based upon One-Way ANOVA analysis and Duncan test at level of significance α = 0.05.

Antifungal activity

The essential oil from the aerial parts of *D. roxburghiana* exhibited the highest zone of inhibition against *A. niger* (17.66±0.57), followed by *A. flavus* (16.66±0.57) and *C. albicans* (16.33±0.57) in the initial screening stage. The MIC was recorded at the lowest concentration of 31.25 µL/mL against all three fungal strains. The results are significant and are comparable to that of the standard (Clotrimazole) used.

Discussion

Uttarakhand is a hilly state and the medicinal plants occurring at higher altitudes are well known for their exceptional medicinal values. *D. roxburghiana* is widely distributed in different regions of Uttarakhand, so, it is quite economical and easy to collect. Research on this plant proves it a good source of phytochemicals. Due to wide availability of this plant, the essential oil from it can be extracted in large quantities which can further be used to separate and collect different important compounds from it. The essential oil of this plant is rich with *β*-panasinsene (11.2%), phytol (10.0%), pentadecanal (8.6%) etc., and several other minor important phytochemicals. Comparing present results with essential oil composition of different parts of the plant of *Ruellia tuberosa* L. of family Acanthaceae from Nigeria it shows, E-phytol (21.06%), tributylacetyl citrate (19.44%) and heptacosane (7.55%) from leaf oil; m-xylene (33.83%), heptacosane (16.57%) and p-xylene (9.6%) from stem oil; tributylacetyl citrate (67.78%) and 2-methyl-2-pentanol (10.15%) from flower oil; heptane (22.25%), heptacosane (12.89%) and borneol (12.48%) from root oil, and, hexacosane (15.43%), sexton (13.12%) and heneicosane (11.14%) from fruit oil[38]. These results are quite different from our results which may be due to different geographic locations of the plants.

Further, the essential oil of *D. roxburghiana* was found significantly potent against bacteria *P. multocida* (15.66±0.57 mm) and *X. phaseoli* (14.33±1.15 mm) as compare to the other bacterial strains under study. Also, the chloroform extract was found significantly active against *X. phaseoli* (16.33±1.15 mm) among four solvent extracts under study. These results indicate that the essential oil have potency to fight well against *P. multocida* and *X. phaseoli*, and, the chloroform extract is rich in phytochemicals which can fight well against *X. phaseoli.* The highest activity of essential oil against fungal strain *A. niger* (17.66±0.57 mm) indicates the oil can be made applicable in infections caused by *A. niger*. The highest DPPH radical scavenging antioxidant assay (82.2%) and the highest Metal chelating antioxidant assay (76.4%) was observed against chloroform extract of the plant *D. roxburghiana* at 100 µg/mL concentration. These results of antioxidant activity indicate that chloroform extract may be a useful source of antioxidants.

Conclusion

This is the first report on essential oil composition from *D. roxburghiana.* The essential oil was a combination of terpenic and non-terpenic compounds. The essential oil from the species showed significant efficacy against bacteria namely, *P. multocida* (15.66±0.57), *X. phaseoli* (14.33±1.15), *K. pneumoniae* (14.00±1.00) and *S. enterica enterica* (13.33±0.57), while, the oil showed significant activity against fungal strain *A. niger* (17.66±0.57). In DPPH antioxidant activity, the chloroform extract

of the plant was observed with significant activity (82.2%) at 100 µg/mL concentration, also, in Metal Chelating assay, chloroform extract was significantly active (74.4%) at 100 µg/mL concentration. The results obtained may be due to the presence of major compounds in the sample, or it may be due to the synergistic effect of various secondary metabolites present. The present study suggests that the plant extracts are a potent source of natural antioxidants, and the essential oil can be used against microbial invasion.

Acknowledgement

The facilities provided by the Advanced Instrumental Research Facility, Jawaharlal Nehru University, New Delhi, India, for GC and GC-MS analyses of the oil samples and the Department of Science and Technology, New Delhi, for infrastructural support (FIST) is highly acknowledged. This research work was financially supported by UGC, New Delhi, India (146261, Dated 01/07/2014).

Conflict of interest

The authors do not have any conflict of interest.

References

1 Mabberley D J, *Mabberley's Plant-Book: A portable dictionary of plants, their classification and uses*, 4th edn, (Cambridge University Press, Cambridge), 2017, vii-xvi.

2 Karthikeyan S, Sanjappa M and Moorthy S, *Flowering plants of India–Dicotyledons. (Acanthaceae – Avicenniaceae)*, (Botanical Survey of India, Kolkata), 2009, 160–164.

3 Strachey R, *Catalogue of the plants of Kumaun and of the adjacent portions of Garhwal and Tibet*, (Periodical Experts, New Delhi), 1974, 134-136.

4 Ssegawa P and Kasenene J M, Medicinal plant diversity and uses in the Sango Bay area, Southern Uganda, *J Ethnopharmacol*, 2007, **113**(3), 521-540.

5 Adeniyi B A and Odufowora R O, *In vitro* antimicrobial properties of *Aspilia Africana* (Compositae), *Afr J Biomed Res*, 2000, **3**(3), 167-170.

6 Telefo P B, Moindipa P E, Tihana A N, Tchouanguep D C and Mbiapo F T, Effects of an aqueous extract of *Aloe buellneri, Justina insular, Hibiscus macranthus, Dicliptera verticillate* some physiological and biochemical parameters of reproduction in immature female rats, *J Ethnopharmacol*, 1998, **63**(3), 225-230.

7 Mbopi P Y, Fozeng H D S, Nguekeu Y M M, Bitchagno G T M, Ngantchouko C B N, *et al.*, Chemical constituents, total phenolic content, antioxidant activity and bactericidal effect of *Dicliptera verticillate* (Acanthaceae), *S Afr J Bot*, 2021, **142**, 216-221.

8 Gaikar P S, ShivankarV S, Patil P A, Chavan A U and Wadhawa G C, Preliminary phytochemical analysis and antioxidant, anti-inflammatory activity of *Dicliptera ghaticas Santapau*, *Int J Aquat Sci*, 2021, **12**(2), 4973-4980.

9 Akbar S, Ishtiaq S, Jahangir M, Elhady S S, Bogari H A, *et al.*, Evaluation of the antioxidant, antimicrobial, and anticancer activities of *Dicliptera bupleuroides* isolated compounds using *In vitro* and *In silico* studies, *Molecules*, 2021, **26**, 7196.

10 Qureshi R, Waheed A, Arshad M and Umbreen T, Medico-ethnobotanical inventory of tahsil Chakwal, Pakistan, *Pak J Bot*, 2009, **41**(2), 529–538.

11 Khan M Z, Khan M A and Hussain M, Medicinal plants used in folk recipes by the inhabitants of Himalayan region Poonch Valley Azad Kashmir (Pakistan), *J Basic Appl Sci*, 2012, **8**, 35–45.

12 Marlesa R J and Farnsworth N R, Antidiabetic plants and their active constituents, *Phytomed*, 1995, **2**(2), 137–189.

13 Bahuguna R P, Jangwan J S, Kaiya T and Sakakibara J, Flavonoids and fatty acids of *Dicliptera roxburghiana*, *Pharm Biol*, 1987, **25**(3), 177–178.

14 Luo Y, Feng C, Tian Y and Zhang G, Glycosides from *Dicliptera riparia*, *Phytochem*, 2002, **61**(4), 449-454.

15 Ghalehnoo Z R, Diseases caused by *Staphylococcus aureus*, *Int J Med Health Res*, 2018, **4**(11), 65-67.

16 Bengoechea J A and Pessoa J S, *Klebsiella pneumoniae* infection biology: living to counteract host defenses, *FEMS Microbiol Rev*, 2019, **43**(2), 123-144.

17 Makvana S and Krilov L R, *Escherichia coli* infections, *Pediatr Rev*, 2015, **36**(4), 167-171.

18 Tsonis I, Karamani L, Xaplanteri P, Kolonitsiou F, Zampakis P, *et al*., Spontaneous cerebral abscess due to *Bacillus subtilis* in an immunocompetent male patient: A case report and review of literature, *World J Clin Cases*, 2018, **6**(16), 1169-1174.

19 Mkangara M, Prevention and control of human *Salmonella enterica* infections: An implication in food safety, *Int J Food Sci*, 2023, **2023**, 1-26.

20 Golob M, Pate M, Kusar D, Dermota U, Avbersek J, *et al.,* Antimicrobial resistance and virulence genes in *Enterococcus faecium* and *Enterococcus faecalis* from humans and retail red meat, *Biomed Res Int,* 2019, **2019**, 12.

21 Ringler D H, Peter G K, Chrisp C E and Keren D F, Protection of rabbits against experimental *Pasteurellosis* by vaccination with a potassium thiocyanate extract of *Pasteurella multocida*, *Infect Immun,* 1985, **49**(3), 498-504.

22 Escobar M A and Dandekar A M, *Agrobacterium tumefaciens* as an agent of disease, *Trends Plant Sci*, 2003, **8**(8), 380-386.

23 Lee Y A, Chen K P and Hsu Y W, Characterization of *Erwinia chrysanthemi*, the soft-rot pathogen of white-flowered calla lily, based on pathogenicity and PCR-RFLP and PFGE analyses, *Plant Pathol*, 2006, **55**(4), 530–536.

24 Zárate-Chaves C A, Cruz D G de la, Verdier V, López C E, Bernal A, *et al*., Cassava diseases caused by *Xanthomonas phaseoli* pv. manihotis and *Xanthomonas cassava*, *Mol Plant Pathol*, 2021, **22**, 1520–1537.

25 Ahmad B, Khan M R, Shah N A and Khan R A, *In vitro* antioxidant potential of *Dicliptera roxburghiana*, *BMC Complement Altern Med*, 2013, **13**, 140.

26 Oyaizu M, Studies on products of Browning reactions: Antioxidative activities of product of Browning reaction prepared from glucosamine, *Jpn J Nutr Diet*, 1986, **44**(6), 307–315.

27 Que F, Mao L, Zhu C and Xie G, Antioxidant properties of Chinese yellow wine, its concentrate and volatiles, *LWT - Food Sci Technol*, 2006, **39**(2), 111–117.

28 Bauer A W, Kirby W M M, Sherris J C and Turek M, Antibiotic susceptibility testing by standardised single disc method, *Am J Clin Pathol*, 1996, **45**(4), 493-496.

29 Afolayan A J, Extracts from the shoot of *Arctotis arctotoides* inhibit the growth of bacteria and fungi, *Pharm Biol*, 2003, **41**(1), 22–25.

30 Prasad R, Bisht L S, Joshi D, Nailwal M K and Melkani A B, Chemical composition and antibacterial activity of the essential oil from whole aerial parts of *Leucas mollissima* Wall. exBenth, *J Essent Oil-Bear Plants*, 2017, **20**(1), 141-147.

31 Bisht L S, Melkani A B, Prasad R, Mohan L, Palni M, *et al.*, Chemical composition and antimicrobial assay of essential oil from whole aerial parts of *Ainsliaea aptera* DC. collected from two different regions of central Himalaya, *J Essent Oil-Bear Plants*, 2021, **24**(3), 510-518.

32 Joshi D, Melkani A B, Nailwal M, Bisht L and Prasad R, *Selinum vaginatum* C. B. Clarke: Terpenoid composition and antibacterial activity of whole aerial parts and root essential oil, *J Essent Oil-Bear Plants,* 2018, **21**(5), 1176-1185.

33 Adams R P, Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn, (Allured Publishing Corporation, Carol stream, Illinois, USA), 2007.

34 Loganayaki N, Siddhuraju P and Manian S, Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L, *J Food Sci Technol*, 2013, **50**(4), 687–695.

35 Yamaguchi F, Ariga T, Yoshimira Y and Nakazawa H, Antioxidant and anti-glycation of garcinol from *Garcinia indica* fruit rind, *J Agric Food Chem*, 2000, **48**(2), 180–185.

36 Gulcin I, Oktay M, Kirecci E and Kufrevioglu O I, Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts, *Food Chem*, 2003, **83**(3), 371–382.

37 Gordon M H, The mechanism of the antioxidant action *in vitro*, edited by B J F Hudson, in *Food Antioxidants*, (London/New York: Elsevier), 1990, 1–18.

38 Moronkola D O, Aboaba S A and Choudhary I M, Composition of volatile oils from leaf, stem, root, fruit, and flower of *Ruellia tuberosa* L. (Acanthaceae) from Nigeria, *J Med Plants Res*, 2015, **8**(41), 1031-1037.