

Investigating the Phytochemical and Bioactive Properties of *Capsicum annuum* var. *cerasiforme* and *Heracleum nepalense*

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ABSTRACT

The fruit of *Capsicum annuum* var. *cerasiforme* belonging to the Family Solanaceae is a widely used spice in Sikkim, a North Eastern state of India. The fruit of *Capsicum annuum* var. *cerasiforme* locally called 'Dalle Khorsani' is a valuable cash crop of Sikkim and also possess medicinal properties. *Heracleum nepalense* D. Don locally called 'Chimphing' belongs to the Family Umbellifereae and is extensively used in folk medicine. The present study evaluated the antimicrobial property of fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don using agar well diffusion method against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis*. The antioxidant property of methanol extracts of fruits of these test plants was also investigated. The antioxidant property was evaluated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity assay and Ferric reducing power assay. The total phenolic content was determined by using Gallic acid as a standard. The methanol extract of both the plants inhibited the growth of mostly Gram positive bacteria and *Escherichia coli* among Gram negative bacteria. However the acetone extract of the selected plants did not exhibit antimicrobial activity. The methanol extracts of these plants exhibited significant ($p < 0.05$) concentration dependent increase in DPPH free radical scavenging activity. The extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish also exhibited significantly higher ($p < 0.05$) ferric reducing ability thereby showing higher antioxidant activity. The phytochemical analyses of the plant extracts revealed the presence of phenol, tannin, flavonoid, alkaloid, steroid and saponin. The results of the present study indicate that *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don can be a potential source of antimicrobial and antioxidant agents and can be explored further for its therapeutic use.

Keywords: Antimicrobial, Antioxidant, *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Heracleum nepalense* D. Don.

INTRODUCTION

Traditional medicines are part of the habitual treatment of various maladies¹. The World Health organization (WHO) has estimated that approximately 80% of the world population relies mainly on plant derived traditional therapies for primary health care². Naturally occurring compounds found in dietary and medicinal plants have been shown to possess antimicrobial and antioxidant activities³. The increasing antibiotic resistance of some pathogens associated with various diseases has encouraged many researchers to search for potent and cost-effective treatment from various sources including plants^{4,5}. The oxidation of biological molecules by toxic Reactive Oxygen Species (ROS) leading to many disease conditions is another concern⁶. Antioxidants and antimicrobial agents from natural sources have gained interest among nutritionists, food manufacturers and consumers, owing to their presumed safety, nutritional and therapeutic properties^{7,8}.

In the present study two plants were selected namely *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don collected from the South district of Sikkim, India. Fruits of these species of plants are widely consumed by the Sikkimese population.

Capsicum annuum var. *cerasiforme* (Red Cherry Pepper) locally called 'Dalle Khorsani' belongs to the Family Solanaceae. *Capsicum annuum* var. *cerasiforme* is one of the valuable cash crops grown all over Sikkim. It is used for making pickle, paste, chilli powder and also possess medicinal properties. *Capsicum annuum* var. *cerasiforme* contains capsaicin, a pungent component responsible for its pungency. Its pungency level is very high with a Scoville rating of 100,000 to 350, 000 SHU (Scoville Heat Units)^{9,10}. 'Dalle khorsani' is also grown in hilly areas of Nepal and Himalayan range and is consumed by local tribal people for curing gastro duodenal diseases¹¹. The beneficial effect of capsaicin in gastrointestinal modulation has been extensively reviewed by Srinivasan (2016)¹². The genus *Capsicum* is a widely used spice in India. It has carminative action and is used as an appetizer. It is sometimes added to tannin or rose gargles for pharyngitis and relaxed sore throat¹³. It is a very good source of vitamins A and C. It is also a source of neutral and acidic phenolic compounds, which are attributed to its antioxidant property¹⁴.

Heracleum nepalense D. Don belonging to the Family Umbellifereae is a small shrub which grows in Nepal and

Sikkim¹⁵. It is locally called 'Chimphing'. The root of *Heracleum nepalense* D. Don is used in folk medicine as digestive, carminative and antidiarrhoeal and is also reported to have antimicrobial and antioxidant activities¹⁶. Antibacterial activity of methanol extract of roots of *Heracleum nepalense* D. Don against bacteria causing diarrhoea has been investigated by Bose et al. (2007)¹⁷. The dried fruits of *Heracleum wallichii* DC. are chewed to treat influenza and sinusitis¹⁸.

The present study evaluated the antimicrobial activity of the methanol and the acetone extracts of fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don. The study also investigated the antioxidant activity of the methanol extract of the test plants.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used including solvents were obtained from Sigma-Aldrich, USA, Merck, Germany and HiMedia, India and were of analytical grade. *Plant material*

The fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish were collected from Lingee Payong and *Heracleum nepalense* D. Don from Damthang, the South district of Sikkim, India. Taxonomic identification of the plant samples was done and the voucher specimens were deposited at the herbarium of the Taxonomy Division, Department of Botany, University of North Bengal, Siliguri, India with accession numbers: *Capsicum annuum* var. *cerasiforme* (Mill.) Irish (09731) and *Heracleum nepalense* D. Don (09732). *Preparation of plant extracts*

Plant samples were air dried at room temperature and powdered using Waring blender (Cole Parmer, RZ-0424521). The powdered materials were extracted with methanol and acetone in Soxhlet apparatus for 24 hours. The solvent was then evaporated under reduced pressure in a Rotary evaporator (Buchi, Switzerland, R-3). The concentrated extract was stored at 4°C until further use¹⁹. Prior to antimicrobial assay, extracts were dissolved in 0.25% dimethyl sulphoxide (DMSO) to obtain various concentrations. *Phytochemical analyses*

The methanol and the acetone extracts of fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don were subjected to phytochemical analyses to examine the phytoconstituents namely phenol, flavonoid, tannin, saponin, steroid, alkaloid, carbohydrate, protein and fat²⁰⁻²³.

Test microorganisms

The test microorganisms were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh and Subhasree Biotech, Kolkata, India. Four Gram negative bacteria: *Klebsiella pneumoniae* (MTCC-3384), *Pseudomonas aeruginosa* (MTCC-1034), *Proteus vulgaris* (MTCC-742), *Escherichia coli* (Subhasree Biotech, Kolkata) and three Gram positive bacteria: *Bacillus cereus* (MTCC- 6840), *Staphylococcus aureus*

(MTCC-7443) and *Bacillus subtilis* (Subhasree Biotech, Kolkata) were used in the study.

Preparation of inoculum

Test microorganisms were maintained at 4°C on nutrient agar slants. Each test bacterial isolates were inoculated into the nutrient broth medium and grown overnight at 37°C. Turbidity was assessed by Spectrophotometer (Lambda 25 UV/Vis /Perkin Elmer, L600-00BB) by measuring the absorbance of the bacterial suspension. The absorbance in the range of 0.08-0.13 OD at 625 nm corresponding to 1×10^8 CFU/ ml (McFarland standard 0.5) was maintained²⁴.

Determination of antimicrobial activity The antimicrobial assay was performed by the agar well diffusion method²⁵. The test plant extracts were dissolved in 0.25% dimethyl sulphoxide (DMSO) to prepare a stock concentration of 100 mg/ml and was sterilized by filtration through 0.45 µm cellulose acetate membrane filter (Sartorius). Various concentrations (10 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml) of methanol and acetone extracts were prepared using the stock concentration. DMSO (0.25%) was used as the negative control. Gentamicin (0.1 mg/ml)²⁶ was used as the positive control. The plates were incubated overnight at 37°C. Antimicrobial activity was determined by measuring the diameter of inhibition zone (DIZ)²⁷ inclusive of the well diameter of 8 mm. The experiment was performed four times and the data presented represent the mean values.

Determination of Minimum Inhibitory Concentration (MIC)

The lowest concentration or highest dilution of the plant extract that inhibits the visible growth of test microorganism is known as minimum inhibitory concentration. The MIC assay was performed by using agar dilution method²⁴. *Determination of antioxidant activity DPPH free radical scavenging assay*

For analysis of antioxidant activity, 0.1 mM DPPH solution was prepared in methanol. In different test tubes, 3 ml of extract at various concentrations (10-100 µg/ml) were added. To each test tube, 1ml of 0.1 mM DPPH in methanol solution was added. The control was prepared in the absence of plant extract. The reaction mixtures were incubated in dark at room temperature for 30 min and the absorbance of the reaction mixtures were taken at 517 nm by using the Spectrophotometer (Lambda 25 UV/Vis /Perkin Elmer, L600-00BB). The decrease in optical density of DPPH on addition of test plant extract in relation to control was used to calculate the antioxidant activity as percentage inhibition (% IP) of DPPH radical²⁸. DPPH Scavanged (%) or % IP

$$= \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})}{(\text{Absorbance}_{\text{control}})} \times 100$$

IC₅₀ values denote the concentration of sample which is 20 minutes. After cooling, aliquot of 10%

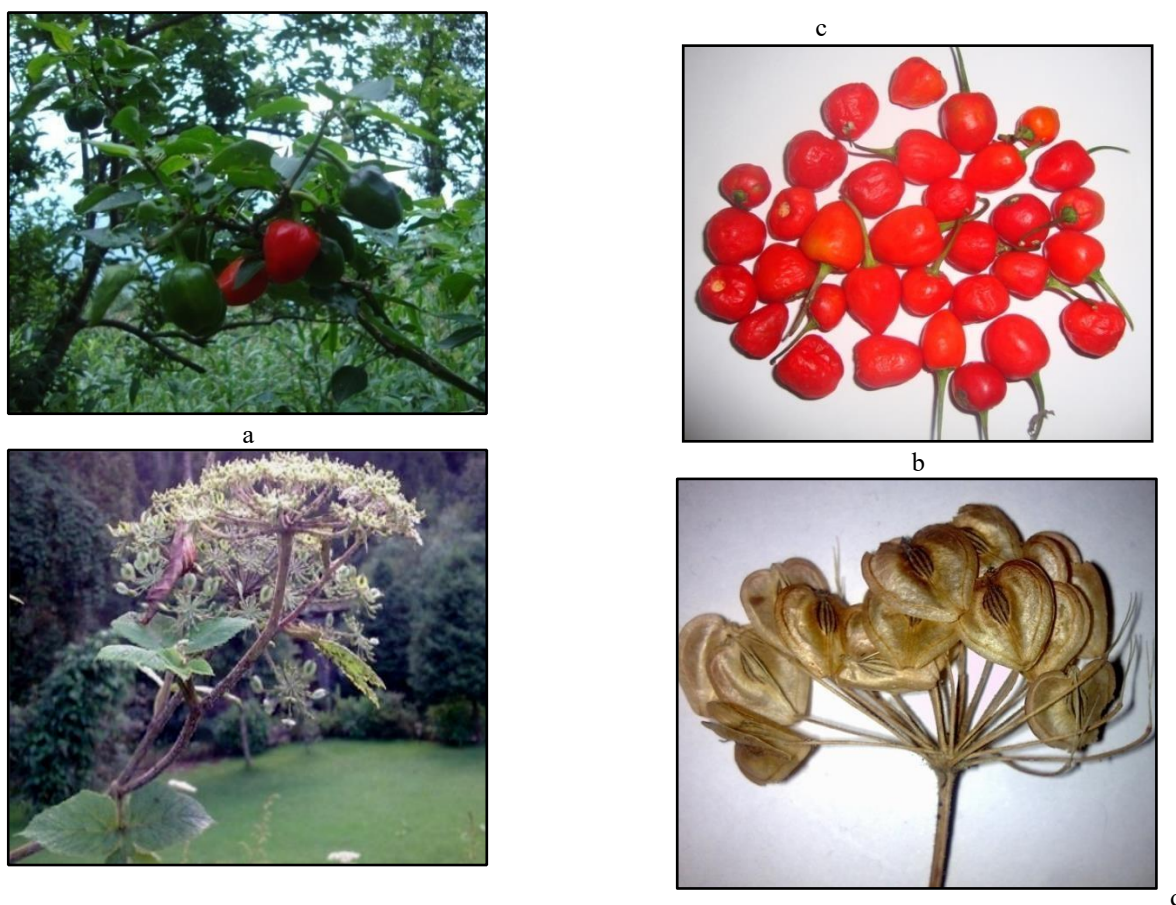


Figure 1: a. *Capsicum annuum* var. *cerasiforme* (Mill.) Irish b. Fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish c. *Heracleum nepalense* D. Don. d. Fruits of *Heracleum nepalense* D. Don.

Table 1: Qualitative phytochemical analysis of methanol and acetone extracts of the test plants.

Phytochemical Tests	<i>Capsicum annuum</i> var. <i>cerasiforme</i> (Mill.) Irish		<i>Heracleum nepalense</i> D. Don	
	Methanol extract	Acetone extract	Methanol extract	Acetone extract
Tannin	-	-	+	-
Phenol	+	-	+	-
Saponin	-	-	+	-
Steroid	+	-	-	-
Flavonoid	+	-	-	+
Alkaloid	+	-	+	-
Carbohydrate	-	-	-	-
Protein and amino acid	-	-	+	-
Fixed oil and fat	+	+	+	+

required to scavenge 50% of DPPH free radicals²⁹. The '+' indicate present; '-' indicate absent.

experiments were performed in triplicate and the data presented represent the mean values.

Reducing power assay

The reducing power of the methanol extracts of test plants was determined by the method of Oyaizu (1986)³⁰. Various concentrations of plant extracts (10-100 µg/ml) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml Potassium ferricyanide (1%). The reaction mixture was kept in water bath at 50°C for

trichloroacetic acid (2.5 ml) was added and centrifuged at 3000 rpm for 10 minutes. After centrifugation the upper layer (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of freshly prepared 0.1% ferric chloride solution. Control was prepared in absence of the plant extract. The absorbance of reaction mixture was measured at 700 nm using the Spectrophotometer (Lambda 25 UV/Vis /Perkin Elmer, L600-00BB).

Ascorbic acid at various concentrations was used as the standard. Increase in the absorbance of reaction mixture indicates an increase in the reducing power of the extract²⁸. The experiments were performed in triplicate and the data presented represent the mean values. *Determination of total phenolic content* Total phenolic content was determined by Folin-Ciocalteu method³¹ using Gallic acid as the standard. To estimate the total phenolic compound

gram of extract. The experiments were performed in triplicate and the data presented represent the mean values.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism V5.01 (San Diego, USA). For antimicrobial assay, data were analysed using one way ANOVA. A value of p<0.05 was considered to indicate a significant increase in the zone of inhibition with the different concentrations of

Table 2: Antimicrobial activity of plant extracts against test microorganisms by agar well diffusion method.

Mean diameter of growth inhibition zone (mm)									
	<i>Escherichia coli</i>		<i>Bacillus cereus</i>		<i>Bacillus subtilis</i>		<i>Staphylococcus aureus</i>		
Concentration (mg/ml)	<i>C. annum</i>	<i>H.nepalense</i>	<i>C. annum</i>	<i>H.nepalense</i>	<i>C. annum</i>	<i>H.nepalense</i>	<i>C. annum</i>	<i>H.nepalense</i>	
100	21.75±0.5*	11	11.25±0.5	13*	18±0.95*	13.75±0.5*	18±0.81*	11.25±0.5	
75	20.25±0.5*	-	-	11.75±0.5	16.75±0.5*	13	17*	-	
50	18.75±0.5*	-	-	12	16.75±0.5*	12±0.0	16±0.81*	-	
25	17.75±0.5*	-	-	11.50±0.5	14.25±0.5	-	14.25±0.5*	-	
10	11.75±0.5	-	-	-	-	-	11.50 ±1	-	
Negative control	-	-	-	-	-	-	-	-	-
Positive control	28.25±0.5		30.25±0.5		31.25±0.5		30.50±0.57		

Each value represents mean ± SD (n=4). '-': absence of zone of inhibition, Negative control: DMSO (0.25%), Positive control: Gentamicin (0.1mg/ml). * p<0.05 Significant increase in the zone of inhibition as compared to the lowest concentration of plant extracts for which the zone of inhibition was observed.

in the plant extracts, 1 ml of *Minimum Inhibitory Concentration (MIC)* Table 3: Minimum Inhibitory Concentration (MIC) in mg/ml of the methanol extracts of *Capsicum annum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don against test microorganisms.

Test microorganisms	<i>Capsicum annum</i> var. <i>cerasiforme</i> (Mill.) Irish	<i>Heracleum nepalense</i> D. Don
<i>Escherichia coli</i>	75	100
<i>Klebsiella pneumoniae</i>	-	-
<i>Pseudomonas aeruginosa</i>	-	-
<i>Proteus vulgaris</i>	-	-
<i>Bacillus cereus</i>	15	50
<i>Bacillus subtilis</i>	15	50
<i>Staphylococcus aureus</i>	50	50

'-'absence of zone of inhibition in agar well diffusion method.

extract was mixed with 1ml Folin-Ciocalteu reagent (10%). After 3 minutes, 3 ml of aqueous sodium carbonate (2%) was added. The reaction mixture was incubated at room temperature for two hours and the absorbance was recorded at 760 nm using Spectrophotometer (Lambda 25 UV/Vis /Perkin Elmer, L600-00BB). The total phenolic content was represented as mg of Gallic acid equivalent (GAE) per

the plant extract. For antioxidant assay, two way ANOVA was performed followed by Bonferroni post-tests. A value of p<0.05 was considered statistically significant.

RESULTS

Fruits of *Capsicum annum* var. *cerasiforme* (Mill.) Irish are widely consumed by the Sikkimese population. *Heracleum nepalense* D. Don is used in folk medicine. The present study investigated the antimicrobial and antioxidant properties of fruits of *Capsicum annum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don collected from the South district of Sikkim, India.

Phytochemical analysis

Table 1 depicted the phytochemical profile of the methanol and the acetone extracts of *Capsicum annum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don.

Antimicrobial activity

The antimicrobial activity of the methanol and the acetone extracts of the test plants were determined by agar well diffusion method against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis*. The extracts of plants showed varying degrees of antimicrobial activity in terms of the mean diameter of growth inhibition zone in millimetre (Table 2). Antimicrobial activities of the

extracts were dependent largely upon the concentration as well as the extraction solvent used in the study. The extract inhibited the growth of mostly Gram positive bacteria and *Escherichia coli* among the Gram negative bacteria. At the concentration of 100 mg/ml the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish showed the maximum zone of inhibition (21.75 ± 0.5 mm) against *Escherichia coli*. For

Heracleum nepalense D. Don, the maximum zone of inhibition (13.75 ± 0.5 mm) was observed against *Bacillus subtilis*. The methanol extracts of both the plants did not inhibit growth of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. For both the plant extracts the MIC value ranged from 15-100 mg/ml (Table

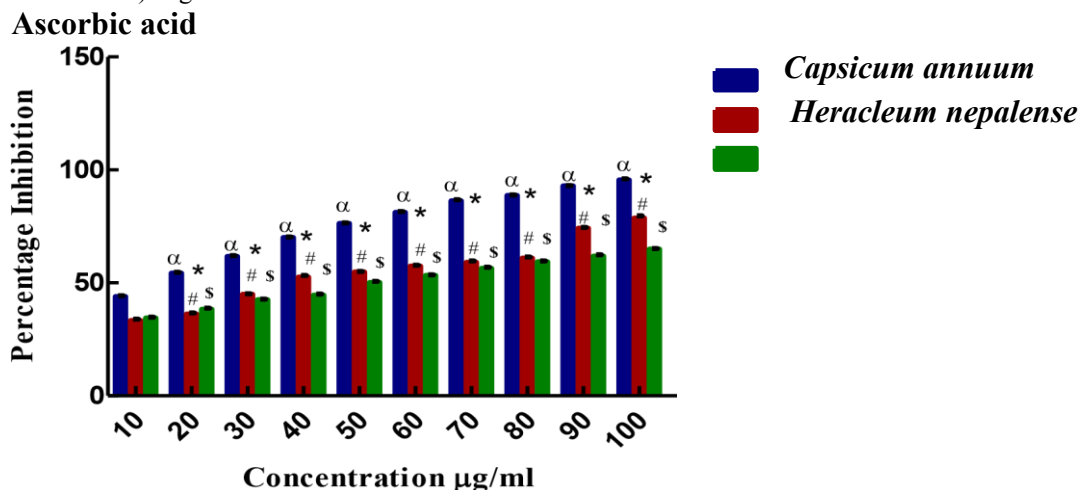


Figure 2: Percentage inhibition of standard (ascorbic acid) and methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don in DPPH scavenging model.

*Significant ($p < 0.05$) difference in free radical scavenging activity of *Capsicum annuum* vs. *Heracleum nepalense*; α, #, \$ significant ($p < 0.05$) concentration dependent increase in free radical scavenging activity of Ascorbic acid, *Capsicum annuum* and *Heracleum nepalense* respectively.

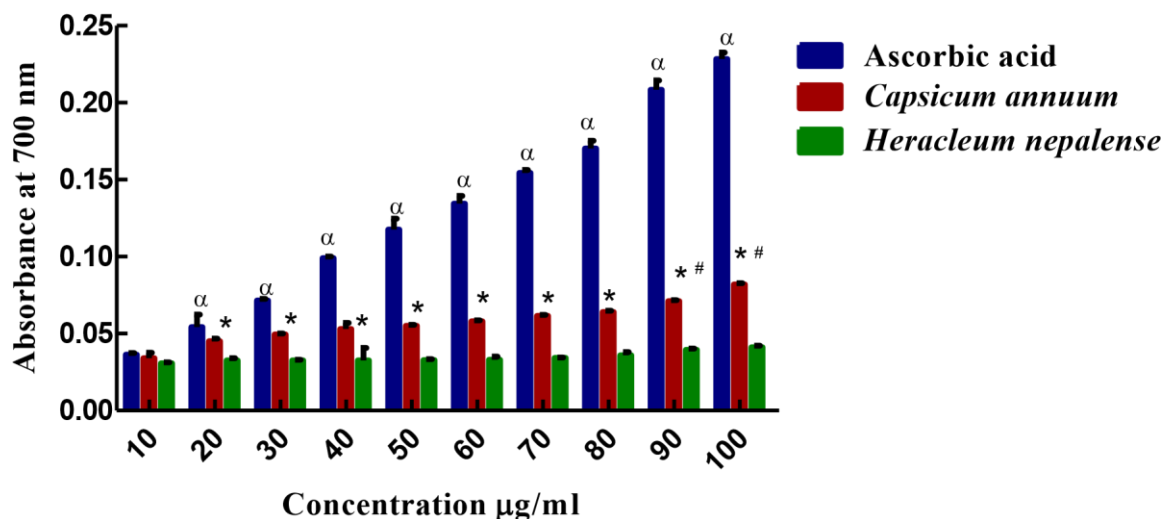


Figure 3: Ferric reducing power of the methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Heracleum nepalense* D. Don. and ascorbic acid (standard).

*Significant ($p < 0.05$) difference in ferric reducing ability of *Capsicum annuum* vs. *Heracleum nepalense*; α, #, significant ($p < 0.05$) concentration dependent increase in ferric reducing ability of Ascorbic acid and *Capsicum annuum* respectively.

3). However the acetone extracts of both the test plants did not inhibit the growth of all the test microorganisms.

Antioxidant activity

The antioxidant activity of the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay and Ferric reducing power assay.

(ascorbic acid) was 20 µg/ml with percentage inhibition of 54.37 ± 0.27 %. The IC₅₀ value of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish was 40 µg/ml with percentage inhibition of 52.55 ± 0.80 % and *Heracleum nepalense* D. Don was 60 µg/ml with percentage inhibition of 53.29 ± 0.27 %.

Reducing power activity

There was significant difference in ferric reducing ability of extracts and ascorbic acid (standard). The methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited concentration dependent increase in reducing power at higher concentrations. The extract showed significantly ($p < 0.05$) higher ferric reducing ability than the methanol extract of *Heracleum nepalense* D. Don (Figure 3).

Total phenolic content

The total phenolic content of the methanol extracts of plants were measured by Folin-Ciocalteu method. The total phenolic content was expressed as mg of GAE/g of extract where GAE represent Gallic acid equivalent. The total phenolic content of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don was found to be 8.81 ± 0.27 mg GAE/g and 8.06 ± 0.11 mg GAE/g respectively.

DISCUSSION

In the present study the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don inhibited the growth of mostly Gram positive test bacteria and *Escherichia coli* among the Gram negative test bacteria (Table 2). Furthermore, it is observed from the result that the MIC values of the methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don for *Escherichia coli* was comparatively higher than the Gram positive test bacteria (Table 3). It has been reported that Gram positive bacteria are more susceptible to plant extracts as compared to Gram negative bacteria³². The present findings were in accordance with the results observed by Rabe and Van Staden (1997) where the test extracts used in their experiments exhibited antimicrobial activity mainly against the Gram-positive bacteria namely *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and against *Escherichia coli* among Gram-negative bacteria³³. This may be attributed to the single layer cell wall structure in Gram positive bacteria as compared to multilayered cell wall structure of Gram negative bacteria^{32,34}. Hence the passage of the bioactive compounds may be inhibited through the Gram

DPPH free radical scavenging activity

The test plant extracts revealed a dose dependent inhibitory effect on DPPH free radical scavenging activity. With an increase in concentration of the plant extracts there was a significant ($p < 0.05$) increase in the DPPH scavenging activity (Figure 2). The IC₅₀ value was defined as the concentration (in µg/ml) of the extract that scavenges the DPPH radicals by 50%. The IC₅₀ value of standard

negative cell wall³⁵. The methanol extracts of both the plants did not exhibit antimicrobial activity against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*. On the other hand, the acetone extract of fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don did not inhibit the growth of all the test microorganisms. It can be speculated that the dose levels or concentrations of the extracts employed in the study may be insufficient to inhibit the growth of these test microorganisms. Lack of antimicrobial activity of the extracts can thus only be proven by using large doses^{35,36}.

The qualitative phytochemical analyses of the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish revealed the presence of phenol, flavonoid, steroid and alkaloid. Similarly, phenol, tannin, saponin and alkaloid were detected in the methanol extract of *Heracleum nepalense* D. Don (Table 1). However, most of the phytochemicals were not detected in the acetone extracts of both the plants which may be attributed to the absence of antimicrobial activity of the extracts against the test microorganisms. Further, methanol has been reported to exhibit better extraction efficiency than other solvents³⁷. The methanol extract of fruits of *Heracleum nepalense* D. Don inhibited the growth of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The extract also exhibited free radical scavenging potential. The fruits and roots of *Heracleum nepalense* is used in folk medicine^{16,18}. The results of the present study may provide a scientific rationale on the basis of the antimicrobial and antioxidant properties for the use of these test plants in the folk medicine.

In the present study, the methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish revealed the presence of phenol and flavonoid. Comparatively higher amount of phenol (8.81 ± 0.27 mg GAE/g) was estimated in the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish. *Capsicum* species have revealed moderate to high levels of neutral phenolics or flavonoids which are the important antioxidant components of a plant based diet¹⁴. It has been reported that the flavonoid present in plants act as antioxidants by stabilising the reactive oxygen species such as superoxide anion, hydroxyl radical or peroxy radicals and may also act as quenchers of singlet oxygen^{38,39}. Antioxidant activity of phenolic compound is attributed to its ability to scavenge free radicals by virtue of their hydrogen donating ability⁴⁰. Furthermore, the fruits of *Capsicum* is one of the richest sources of carotenoids, Vitamin C and Vitamin E which are

considered as the most powerful antioxidant substances which provides profound protection against oxidative agents⁴¹. Capsaicinoids including capsaicin and dihydrocapsaicin, the pungent components present in fruits of *Capsicum* also contributes to the antioxidant activity^{14,42}. Hence the strong antioxidant activity of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish may be due to the presence of these phytochemicals. The roots of *Heracleum nepalense* have been reported to contain coumarins, steroids and flavonoids¹⁶. However in the methanol extract of fruits of *Heracleum nepalense* D. Don, flavonoid was not detected which may possibly relate to the relatively lower antioxidant activity of *Heracleum nepalense* D. Don as compared to the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish. The presence of phenolic compounds along with other phytochemicals could be responsible for the observed antimicrobial and antioxidant properties of the test plant extracts. Spices and aromatic plant materials have long been used in food as flavour, fragrance, appetizer as well as due to their preservative and medicinal properties⁴³. The results of the present study revealed potential antimicrobial and antioxidant properties of the methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don.

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