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STUDIES ON ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *CALTHA PALUSTRIS* L.

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Abstract

Antibacterial and antioxidant activities of *Caltha palustris* L. were evaluated by using agar-well diffusion method and DPPH free radical scavenging assay respectively. Antibacterial activity of different plant part extracts in acetone and methanol solvents was tested against four human pathogenic bacteria namely *Escherichia coli, Listeria monocytogenes, Shigella dysenteriae* and *Staphylococcus aureus* using different concentration (i.e. 25, 50, 75 and 100%.) Further, antioxidant activity of different extracts in acetone and methanol was also studied at 5%, 10%, 15% and 20% µg/mL concentrations. Extracts of different plant parts exhibited significant antibacterial and antioxidant activities. Methanol root extract showed greater zone of inhibition as compared to others extracts. Similarly, acetone leaf extract of this plant showed greater zone of inhibition as compared to others. Root and leaf acetone extracts of this plant exhibited higher antioxidant potential with lowest IC50 values 33.43 and 43.83 respectively. Potential antibacterial and antioxidant activity of this plant further investigated for knowing the bioactive molecules.

Key words: Zones of inhibition, Plant extracts, antibacterial activity, agar-well diffusion methods, DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay.

Introduction

India is one of the 17-mega biodiversity centres possessing 10% of the biodiversity wealth in the world (Shiva, 1996). There are around 20,000 species of medicinal plants recorded in India till today (Dev, 1997). Himachal Pradesh is a hub of medicinal plants and people of the state have a good faith in curative properties of medicinal plants (Sharma *et al*., 2014). In India, different parts of medicinal plants have been used to cure various diseases from time immemorial (Bhattacharjee, 1998). Since, most of the available antimicrobial drugs are being resisted by microorganisms therefore researchers have focused on screening novel bioactive compounds from various medicinal plants having potential to overcome multiple drug resistance and be effectively curative in nature (Bizauyehu and Assefa, 2017). Medicinal plants produce a diverse range of bioactive molecules making them source of many useful drugs (Sukanya *et al*., 2009). Antioxidants protect the bio-molecules and different cell components from harmful free radicals by various mechanisms (Braca *et al*., 2002). The antioxidant compounds are effective free radical scavengers and popularly called secondary metabolites, present in all parts of plants (Methew and Abraham, 2006). These secondary metabolites functions as singlet or triplet oxygen quenchers, enzyme inhibitors, peroxide decomposers and synergists (Larson, 1998). Natural antioxidants are in more use as compared to synthetic antioxidants because these are less toxic, cost effective and having no side effects. Therefore interest in natural antioxidants is growing rapidly. Certain phytochemicals like flavonoids and phenolics are potent for free radical scavenging activity (Channda and Dave, 2009). Investigated medicinal plant *Caltha palustris* L. is a wild species of Ranunculaceae family having ethno-botanical medicinal uses has been collected and analised from district Kullu of Himachal Pradesh.

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Materials and Methods

Collection of plant material

Kharidhar is the name of collection area of this plant (*C. palustris*) located at altitudinal range of 2500-3500 meters in Panchayat Picchalihar, Tehsil and District Kullu

(H.P.), India. Plant samples were collected in the Months of June and July.

Processing of plant material

All the plant parts used (root and leaf) were washed under running tap water and then surface sterilised with 2% mercuric chloride. All parts were then allowed to shade dried for 20-30 days. Dried plant materials were then crushed to make fine powder with the help of mortal-pestle. Prepared fine powders were kept at room temperature in air tight containers.

Preparation of acetone and methanol extracts of different parts of the plant

Five grams of dried roots and leaves of *C. palustris* were taken in three different Erlenmeyer flasks to which 50 mL acetone and methanol were added, followed by covering the flasks with aluminium foils. Flasks were allowed to stand for 5 days for extraction purposes. After extraction, the extracts were filtered with the Whattman filter paper no. I and evaporation was done with the help of rotary evaporator at 40^{0}C. Dried extracts were collected and weighed to prepare stock solutions of conc. 50 mg/mL.

Procurement of bacteria

Different strains of bacteria (*Staphylococcus aureus, Escherichia coli, Shigella dysenteriae* and *Listeria monocytogenes*) used for antibacterial studies were procured from IGMC, Shimla and Department of Biotechnology, HPU, Shimla for screening antibacterial properties of different plant extracts.

Revival of pathogens

The revival of collected bacterial pathogens was done in nutrient broth and storage was done in nutrient agar slants at 4^{o}C.

Screening of different extracts of *C. palustris* for antibacterial activity

Different extracts (acetone and methanol) of *C. palustris* were investigated using Agar-well diffusion method. Nutrient agar medium (Distilled water 1 L, Agar 20 g , Peptone 5g , Sodium Chloride 1g , Yeast extract 2g , Beef extract 1g) was taken throughout the investigation. Autoclaving at 121.6^{o}C for 30 minute was done to sterilize the medium. Bacteria were allowed to grow in nutrient broth for 24 hours. A bacterial suspension of 100 µL was spread on each solidified agar plate. Five agar wells of 7 mm were made in each Petri plate with the help of sterilized stainless steel borer. Four wells were loaded with 25%, 50%. 75% and 100% concentration of prepared plant extracts in each Petri plate and 5th well in centre of the Petri plate was taken as control, contained pure solvent (acetone/ methanol) only. The plates were incubated at 37±2^{o}C for 24 hours in incubation chamber. Antibacterial activities were measured as zone of inhibition (Z.O.I.) of bacterial growth around the well including the well diameter. Measurements were taken in perpendicular direction in

Table 1.1: Antibacterial activity shown by streptomycin (5µg/ mL) used as control (Fig. A).

Test Bacteria	Zone of inhibition (mm) including well
S. dysenteriae	29
S. aureus	26
E.coli	26
L. monocytogenes	27

Table 1.2: Antibacterial activity of different extracts of root of *C. palustris* against different pathogenic bacteria (Figs. B-C).

Extract	Conc. (%)	Inhibition zone diameter in mm (±S.E.)			
		S. aureus	***L. monocytogenes***	***E. coli***	***S. dysenteriae***
Root extract (acetone)	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	12.33±0.27	12.67±0.27	11.33±0.27	11.67±0.27
	50	13.67±0.27	13.33±0.27	13.33±0.27	13.33±0.27
	75	15.33±0.27	14.67±0.27	15.33±0.27	14.67±0.27
	100	16.67±0.27	18.33±0.27	20.67±0.27	15.67±0.27
Root extract (MeOH)	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	11.00±0.47	11.00±0.00	10.33±0.27	13.00±0.47
	50	13.00±0.00	14.33±0.27	11.33±0.27	14.67±0.27
	75	16.33±0.27	15.67±0.27	12.00±0.47	18.67±0.27
	100	19.00±0.00	18.33±0.27	15.33±0.27	23.67±0.27

Each data point represents mean of three replicates ± S.E. (Standard Error)

Table 1.3: Antibacterial activity of different extracts of leaf of *C. palustris* against different pathogenic bacteria (Figs. D-E).

Extract	Conc. (%)	Inhibition zone diameter in mm (±S.E.)			
		S. aureus	***L. monocytogenes***	***E. coli***	***S. dysenteriae***
Leaf extract (acetone)	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	12.67±0.27	18.67±0.27	14.00±0.47	11.00±0.00
	50	15.00±0.00	20.67±0.27	18.00±0.47	13.00±0.47
	75	19.33±0.27	22.67±0.27	20.67±0.27	18.00±0.47
	100	21.67±0.27	25.33±0.27	24.33±0.27	21.33±0.27
Leaf extract (MeOH)	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	11.00±0.47	12.00±0.00	14.33±0.54	10.00±0.00
	50	13.00±0.47	14.33±0.27	15.33±0.27	12.67±0.27
	75	16.00±0.47	15.67±0.27	16.67±0.27	15.00±0.47
	100	18.67±0.27	18.67±0.27	19.00±0.00	17.67±0.27

Each data point represents mean of three replicates ± S.E. (Standard Error)

all the three replicates and average values were tabulated. Streptomycin was used as a standard (Prakash *et al*., 2016; Rana *et al*., 2016).

Screening of different extracts of *C. palustris* for antioxidant activity

The percent free radical scavenging activities of plant extracts were measured by using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) as described by Blois (1958) with slight modifications. 1 mL of different concentrations *viz.,* 5, 10, 15, 20 and 25 µg/mL plant extracts, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank samples of ascorbic acid were used as reference standard and mixture of 1 mL DPPH solution and 1 mL methanol (without plant extract) was used as control. All the tests were carried out in triplicates and decrease in absorbance was measured at 517 nm after 30 minutes in

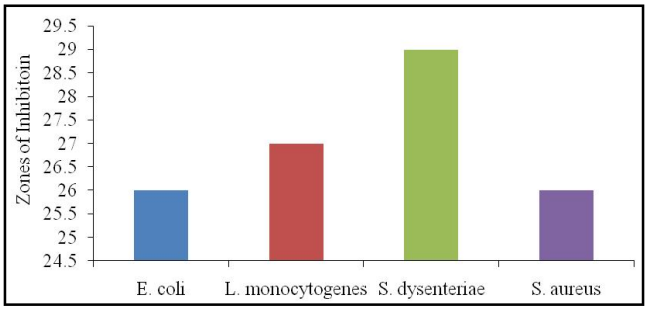

Fig. A: Histograms showing antibacterial activity showed by streptomycin (5 µg/mL) used as control.

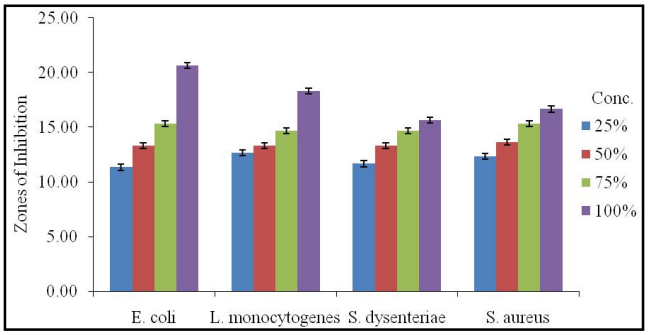

Fig. B: Histograms showing antibacterial activity of acetone root extract of *C. palustris* against different pathogenic bacteria.

Table 2.1: Percent (%) free radical scavenging activity of ascorbic acid (used as standard) at different concentration in methanol (Fig. F).

Ascorbic acid (control)	Concentration (µg/mL)	Free Radical Scavenging Activity (%)	IC_{50} Value (µg/mL)
	5	47.80±0.006	5.84
	10	53.65±0.004	
	15	59.08±0.001	
	20	61.37±0.002	
	25	63.13±0.001	

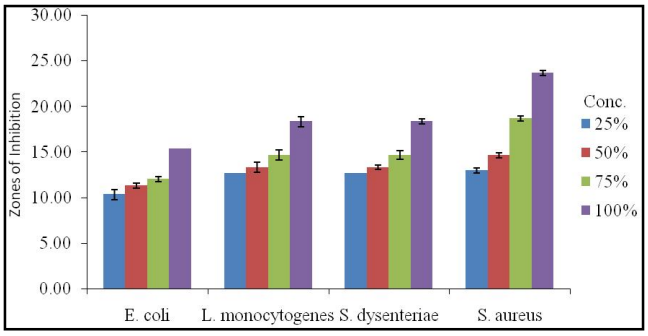

Fig. C: Histograms showing antibacterial activity of methanol root extract of *C. palustris* against different pathogenic bacteria.

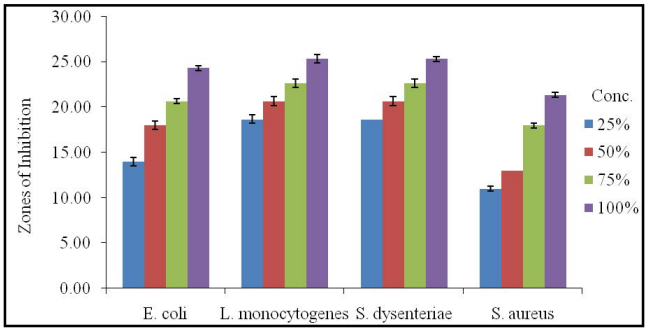

Fig. D: Histograms showing antibacterial activity of acetone leaf extract of *C. palustris* against different pathogenic bacteria.

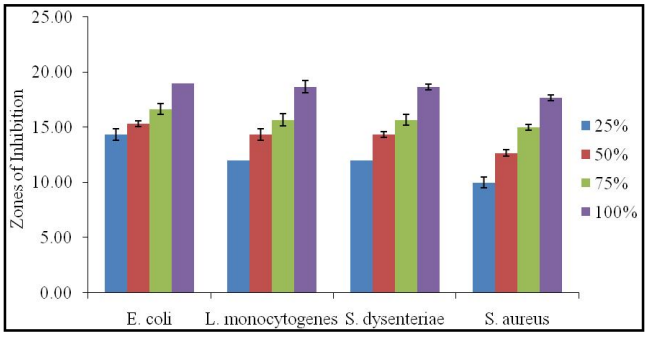

Fig. E: Histograms showing antibacterial activity of methanol leaf extract of *C. palustris* against different pathogenic bacteria.

Table 2.2: Percent (%) free radical scavenging activity of leaf extract of the *C. palustris* at different concentrations in acetone and methanol (Fig. G).

Name of the plant	Concentration (µg/mL)	Acetone extract	IC_{50} Value (µg/mL)	Methanol extract	IC_{50} Value (µg/mL)
C. palustris (leaf)	5	2.88±0.005	43.83	5.25±0.012	73.58
	10	3.49±0.003		10.32±0.007	
	15	14.81±0.007		14.49±0.009	
	20	19.54±0.006		15.94±0.003	
	25	26.54±0.009		18.29±0.005	

dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

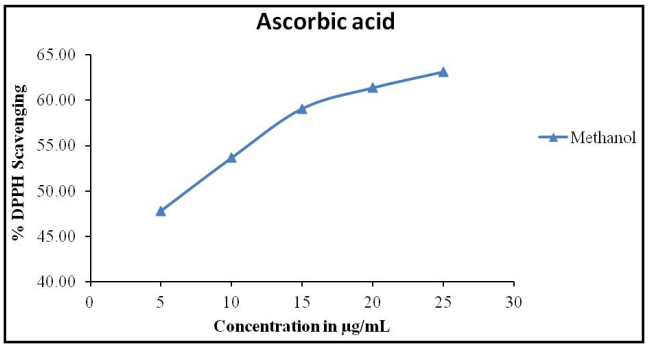

Fig. F: Percent (%) free radical scavenging activity of ascorbic acid (used as standard) at different concentration in methanol.

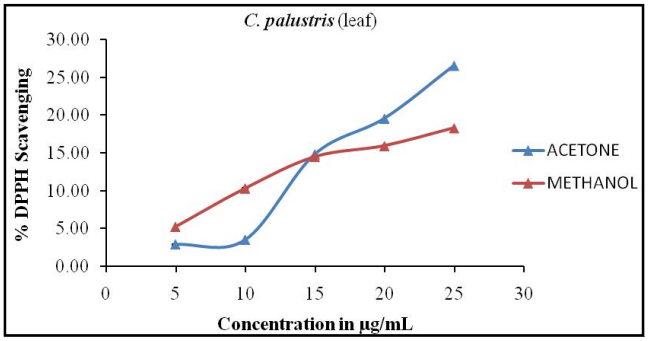

Fig. G: Percent (%) free radical scavenging activity of leaf extract of the *R. australe* at different concentrations in acetone and methanol.

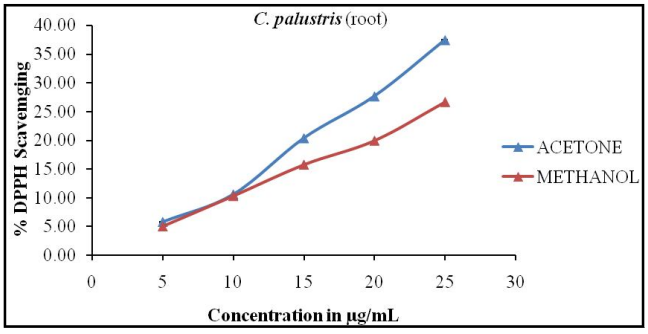

Fig. H: Percent (%) free radical scavenging activity of root extract of the *R. australe* at different concentrations in acetone and methanol.

DPPH scavenging effect (%) =

$$\left(\frac{Acontrol - Asample}{Acontrol}\right)\times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample.

Graphs were plotted against percent inhibition v/s conc. of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation

$$IC_{50} = \frac{50 - Y - Intercept}{Slope}$$

Results

Screening of different extracts of *C. palustris* for antibacterial activity

Table 1.1 and Fig. A showed control (Z.O.I. by streptomycin at 5 µg/mL) against all the four tested bacteria. Tables 1.2, 1.3 and Figs. B-E highlighted the results of antibacterial activities of leaf and root extracts of *R. australe* showing gradual increase in zone of inhibition (Z.O.I.) with increasing concentrations against all the tested bacteria. Results from table 1.2 concluded that acetone root extract shows remarkable antibacterial activity with maximum Z.O.I. (20.67 mm) against *E. coli* and minimum Z.O.I. (11.33 mm) against same bacterium i.e. *E. coli*. Methanol root extract also shows remarkable antibacterial activity with maximum Z.O.I. (23.67 mm) against *S. dysenteriae*. As per table 1.3, acetone leaf extract showed maximum Z.O.I. (25.33 mm) against *L. monocytogenes* and minimum Z.O.I. (11.00 mm) against *S. dysenteriae*. Methanol leaf extract shows maximum Z.O.I. (19.67 mm) against *E. coli.* and minimum Z.O.I. (10.00 mm) against *S. dysenteriae.*

Screening of different extracts of *C. palustris* for antioxidant activity

Table 2.1 and Fig. F showed ascorbic acid taken as standard (0.1 Mm in methanol) exhibiting IC_{50} of 5.84 µg/mL. Tables 2.2-2.3 and Figs. G-H shows antioxidant activity of acetone and methanol extracts of different parts (leaf and root) of *C. palustris*. Table 2.2 and Fig. G shows maximum antioxidant activity (%) in acetone leaf extract with IC_{50} value of 43.83 µg/mL and minimum antioxidant activity (%) in methanol leaf extracts with IC_{50} value of 73.58 µg/mL.

Table 2.3: Percent (%) free radical scavenging activity of root extract of the *C. palustris* at different concentrations in acetone and methanol (Fig. H).

Name of the plant	Concentration (µg/mL)	Acetone extract	IC_{50} Value (µg/mL)	Methanol extract	IC_{50} Value (µg/mL)
C. palustris (root)	5	5.76±0.011	33.43	5.07±0.012	47.38
	10	10.56±0.001		10.33±0.007	
	15	20.35±0.007		15.76±0.009	
	20	27.64±0.009		19.93±0.003	
	25	37.43±0.023		26.63±0.005	

Table 2.3 and Fig. H shows free radical scavenging activity (%) of acetone (IC_{50} 33.43 µg/mL) and methanol (IC_{50} 47.38 µg/mL) root extracts.

Discussion

Antibacterial activities of different extracts of *C. palustris*

Antibacterial activity of leaf and root extracts of *C. palustris* was investigated using acetone and methanol extracts at different concentrations. In case of root, methanol extract showed slightly greater Z.O.I. (23.67 mm) against *S. dysenteriae* as compared to acetone root extract (20.67 mm) against *E. coli* at 100% concentration but it was opposite in case of leaf extract *i.e.* acetone leaf extract showed greater Z.O.I. (25.33 mm) against *L. monocytogenes* as compared to methanol leaf extract (19.00 mm) against *E. coli* at 100% concentration.

Antibacterial activity of 28 plants including *C. palustris* were screened and evaluated against *E. coli* by NandaKafle *et al.,* (2015). In this study also *Caltha palustris* showed significant antibacterial activity. Considerable antibacterial potential has been reported in *C. palustris* from J & K by Mubashir *et al.,* (2014).

Antioxidant activities of different extracts of *C. palustris*

Free radical scavenging activity (%) of acetone leaf extract of *C. palustris* was 43.83 µg/mL (IC_{50}) and it was 73.58 µg/mL (IC_{50}) for methanol leaf extract. Free radical scavenging activity (%) of acetone and methanol root extracts was 33.43 µg/mL (IC_{50}) and 47.38 µg/mL (IC_{50}) respectively. Mubashir *et al.,* (2014) have reported 55.58% DPPH as free radical scavenging *C. palustris* in their study.

Conclusions

It can be concluded from the present study that *C. palustris* exhibited considerable antibacterial and antioxidant potential which can be attributed to the presence of different phytochemicals in its leaves and roots. This potential can be attributed to different bioactive compounds present in this plant and they also needed to be isolated and purified in further studies.

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