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ANTIBACTERIAL AND ANTIOXIDANT POTENTIALOF A MEDICINAL PLANT *RHEUM AUSTRALE* D. DON

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ABSTRACT

Rheum australe D. Don is a higher altitudinal (2500-3300 meters) medicinal plant. It belongs to family Polygonaceae. Extracts of roots and leaves of *R. australe*in acetone and methanol were studied for antioxidant and antibacterial potentials. Antibacterial potentialwas investigated with the use using Agar-Well Diffusion technique against four different strains of bacteria *i.e.*, *Staphylococcus aureus, Shigella dysenteriae, Listeria monocytogenes* and *Escherichia coli* at various conc. as of 25%, 50%, 75% and 100%. Streptomycin (5µg/mL) was taken as a standard or control. Maximum value of Z.O.I. in extracts of roots was found 25.33 mm for acetone root extract, against all the four test bacteria and maximum Z.O.I. for methanol root extract was found 25.67 mm, for*S. aureus* and *E. coli*. Maximum value of Z.O.I. in leaf extracts was found for acetone leaf extract *i.e.*, 19.67 mm against *L. monocytogenes* and maximum Z.O.I. for methanol leaf extract was 15.33 mm against *S. aureus.* Antioxidant activities were also determined by employing DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay at various concentrations *viz.*, 5, 10, 15, 20 and 25 µg/mL. Ascorbic acid was taken as standard with IC_{50} value of 5.84 µg/mL. Highest antioxidant activity was shown by methanol leaf extract with IC_{50} value of 20.37 µg/mL and lowest antioxidant activity was shown by acetone leaf extracts with IC_{50} value of 27.12µg/mL. The results indicate the significant antibacterial and antioxidant activities of tested plant extracts which is the base for future exploitation and utilization of *R. australe* as a natural antioxidant and antibacterial agent.

***Key words*:** *Rheum australe* D. Don, Agar-well diffusion method, DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay.

Introduction

Recently, the most challenging task in medical science is an emergence of multiple drug resistance of various pathogens and it is mostly a consequence of an imprudent use of synthetic antibiotic drugs for the treatment of infectious diseases, hence it is an immediate need to search for various medicinal plants containing new antimicrobial substances (Service, 1995). Because microorganisms are resistant to the majority of available antimicrobial drugs, researchers have concentrated on screening new bioactive compounds from a variety of medicinal plants that may be able to overcome multiple drug resistance and have a curative effect (Bizauyehu and Assefa, 2017). The pathogenic microbes are exhibiting an increased antibiotic resistance that necessitated screening of several medicinal plants for their potential antimicrobial activity (Martins *et al.*, 2001). An antimicrobial substance is a bioactive compound that inhibits the growth of microorganisms such as fungi, bacteria, viruses and protozoa (McEwen and Fedorka-Cray, 2002).

The biochemical compounds preventing or reducing the oxidative reactions being catalyzed by the free radicals inside the body are known as antioxidants and these antioxidants do so by counterbalancing reactive oxygen species (ROS) by donation of hydrogen atom (Erkan *et al.*, 2008). Oxygen is essential for life but at the same time it has a highly toxic nature due to its potential of generating free radicals that leads to chromosomal, lipid, protein and DNA damage by mutations. Although animal cells exhibit an elaborate defense mechanism for free radical detoxification by number of antioxidant enzymes

Table 1: Screening of antibacterial activity shown by streptomycin (5µg/mL) used as control.

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

i.e., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), medicinal plants-based antioxidant compounds increase the antioxidant capacity of plasma further that lowers the risk of certain diseases (Prior and Cao, 2000).

The investigated medicinal plant *R. australe* D. Don belongs to family Polygonaceae and has been used as a potent anti-inflammatory herbal medicine. Different plant parts are also used in abdominal pain, appetite, asthma, bronchitis, fever, cuts, dysentery, laxative, eye diseases, sprain, swelling, ulcer, wounds laxative, stomachic and sperients. Powdered rhizomes are sprinkled over ulcers for quick healing (Rana *et al.*, 2011). Antibacterial activity of *R. australe* was investigated by using Agar-well diffusion method against four human pathogenic bacteria *i.e.*, *Staphylococcus aureus, Shigella dysenteriae, Listeria monocytogenes* and *Escherichia coli* and antioxidant activity was determined by using DPPH free radical scavenging assay.

Materials and Methods

Collection of plant materials

Kharidhar refers to the designated area for collecting test plants (*R. australe*) situated at an altitude between 2500 and 3500 meters in Panchayat Picchalihar, Tehsil, and District Kullu (H.P.), India. Samples of the plant were collected during the months of June and July.

Processing of plant material:

All used plant components (root and leaf) were rinsed under continuously flowing tap water and subsequently disinfected with 2% mercuric chloride. After this, all parts were left to air dry in the shade for 20-30 days. The dried plant materials were then crushed into a fine powder using a mortar and pestle. The resulting fine powders were stored at room temperature in sealed or airtight containers.

Preparation of acetone and methanol extracts:

Five grams of the roots and leaves of *R. australe* were placed in three separate Erlenmeyer flasks, to which 50 mL of acetone and methanol were added. The flasks were then covered with aluminum foil and left to stand for five days to facilitate the extraction process. After the extraction, the mixtures were filtered using Whatman filter paper no. I, and the solvent was removed using a rotary evaporator set at 40°C. The resulting dried extracts were collected and weighed in order to prepare stock solutions with a concentration of 50 mg/mL.

Processing of bacteria:

Various bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, and *Listeria monocytogenes*, were obtained from IGMC, Shimla, and the Department of Biotechnology at HPU, Shimla, for the purpose of evaluating the antibacterial properties of different plant extracts.

Revival of pathogens

The revival of preserved bacterial pathogens was carried out in nutrient broth, while storage was maintained in nutrient agar slants at 4°C.

Screening of antibacterial activity of different extracts of *R. australe*:

Different extracts (acetone and methanol) from *R. australe* were examined using the Agar-well diffusion method. The nutrient agar medium consisted of 1 L of distilled water, 20 g of agar, 5 g of peptone, 1 g of sodium chloride, 2 g of yeast extract, and 1 g of beef extract

Table 2: Screening of antibacterial activity of different extracts of LEAF of *R. australe*.

Extracts	Conc. (%)	Inhibition Zone Diameter in mm (±S.E.)			
		S. aureus	*L. monocytogenes*	*E. coli*	*S. dysenteriae*
R. australe leaf extract (acetone)	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	09.67±0.27	**10.33±0.27**	08.33±0.27	08.33±0.27
	50	10.67±0.27	**14.33±0.27**	13.67±0.27	10.67±0.27
	75	14.33±0.27	**16.67±0.27**	14.67±0.27	14.67±0.27
	100	18.33±0.27	**19.67±0.27**	16.67±0.27	16.33±0.27
R. australe leaf extract (MeOH)	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	**08.67±0.27**	**08.67±0.27**	**08.67±0.27**	**08.67±0.27**
	50	09.67±0.27	**11.67±0.27**	09.67±0.27	09.33±0.27
	75	12.33±0.27	**12.67±0.27**	11.67±0.27	10.33±0.27
	100	**15.33±0.27**	14.33±0.27	13.33±0.27	11.33±0.27

Table 3: Screening of antibacterial activity of different extracts of ROOT of *R. australe*.

Extracts	Conc. (%)	Inhibition Zone Diameter in mm (±S.E.)			
		S. aureus	***L. monocytogenes***	***E. coli***	***S. dysenteriae***
R. australe root extract (acetone)	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	19.67±0.27	**20.33±0.27**	17.33±0.27	19.67±0.27
	50	20.67±0.27	**21.33±0.27**	18.67±0.27	**21.67±0.27**
	75	22.33±0.27	**23.67±0.27**	**23.67±0.27**	22.67±0.27
	100	**25.33±0.27**	**25.33±0.27**	**25.33±0.27**	**25.33±0.27**
R. australe root extract (MeOH)	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	**19.67±0.27**	18.67±0.27	18.33±0.27	13.33±0.27
	50	**21.67±0.27**	19.67±0.27	20.33±0.27	15.33±0.27
	75	**23.33±0.27**	20.67±0.27	21.67±0.27	17.33±0.27
	100	**25.67±0.27**	24.33±0.27	**25.67±0.27**	21.33±0.27

throughout the study. The medium was sterilized by autoclaving at 121.6°C for 30 minutes, after which it was poured into Petri dishes. Bacteria were cultured in nutrient broth for duration of 24 hours. A bacterial suspension of 100 µL was evenly spread across each solidified agar plate. Using a sterilized stainless-steel borer, five wells measuring 7 mm were created in each Petri dish. Four wells in each plate were filled with 25%, 50%, 75%, and 100% concentrations of the prepared plant extracts, while the fifth well in the center served as a control that contained only the pure solvent. The plates were kept in an incubation chamber at 37±2°C for 24 hours. Antibacterial activity was assessed by measuring the zone of inhibition (Z.O.I.) of bacterial growth surrounding the well, including the well's diameter. Measurements were taken in perpendicular directions across all three replicates, and the average values were recorded. Streptomycin was utilized as a standard (Prakash *et al.*, 2016; Rana *et al.*, 2016).

Screening of antioxidant activity of different extracts of *R. australe*:

The scavenging activities of free radicals in plant extracts were evaluated using DPPH (2,2-Diphenyl-1-picrylhydrazyl) according to the method described by Blois (1958), with minor adjustments. Different concentrations of plant extracts (5, 10, 15, 20, and 25 µg/mL) were prepared, and 1 mL of a 0.1 mM DPPH solution in methanol was added to each. Ascorbic acid served as a reference standard for the corresponding blank samples, while a control was created using a mixture of 1 mL of DPPH solution and 1 mL of methanol (without plant extract). All tests were conducted in triplicate, and the reduction in absorbance was measured at 517 nm after a 30-minute incubation in the dark using a UV-VIS spectrophotometer. The inhibition percentage was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where,

A control is the absorbance of control;

A sample is the absorbance of sample.

Graphs were created with percent inhibition plotted against the concentration of both plant extracts and the standard ascorbic acid to determine the slope and y-intercept values. The IC_{50} value, representing the quantity of antioxidant needed to reduce the initial DPPH concentration by 50%, was calculated for each extract and ascorbic acid using the appropriate equation.

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

Results

Screening of antibacterial activity of different extracts of *R. australe*

Table 1, shows the results of antibacterial activity of streptomycin (5µg/mL, used as control) against all the four tested bacteria in terms of zone of inhibition (Z.O.I.) *i.e.*, *S. dysenteriae* (29 mm), *S. aureus* (26 mm), *E. coli* (26 mm), and *L. monocytogenes* (27 mm). As shown in Table 2 and Table 3, antibacterial activities of leaf and root extracts of *R. australe*are showing gradual increase in zone of inhibition (Z.O.I.) with increasing concentrations against all the tested bacteria. As per Table 2, acetone leaf extract showed maximum Z.O.I.

Table 4: Percent (%) free radical scavenging activity of ASCORBIC ACID (used as standard) at different concentration in methanol.

	Conc. (µg/mL)	Free radical scavenging activity (%)	IC_{50} Value (µg/mL)
Ascorbic acid (Control)	5	47.80±0.006	**5.84**
	10	53.56±0.004	
	15	59.08±0.001	
	20	61.37±0.002	
	25	63.13±0.001	

Table 5: Percent (%) free radical scavenging activity of LEAF extract of *R. australe* at different concentration in acetone and methanol.

Plant part	CO	Acetone Extract	IC	Methanol Extract	IC
***R. australe* (leaf)**	5	12.96±0.003	**27.12**	25.47±0.009	**20.37**
	10	20.58±0.003		35.31±0.003	
	15	27.98±0.010		42.28±0.002	
	20	38.68±0.011		50.59±0.010	
	25	46.50±0.004		55.59±0.006	
CO: Conc. (µg/mL), **IC:** IC_{50} Value(µg/mL)					

Table 6: Percent (%) free radical scavenging activity of ROOT extract of *R. australe* at different concentration in acetone and methanol.

Plant part	CO	Acetone Extract	IC	Methanol Extract	IC
***R. australe* (root)**	5	23.25±0.005	**23.55**	23.83±0.012	**25.21**
	10	28.40±0.003		33.39±0.007	
	15	35.54±0.007		38.61±0.009	
	20	39.30±0.006		43.13±0.003	
	25	46.30±0.009		49.22±0.005	
CO: Conc. (µg/mL), **IC:** IC_{50} Value(µg/mL)					

(19.67 mm) against *L. monocytogenes* and minimum Z.O.I. (08.33 mm) against *E. coli* and *S. dysenteriae.* Methanol leaf extract showed maximum Z.O.I. (15.67 mm) against *S. aureus* and minimum Z.O.I. (08.67 mm) against S. *aureus, E. coli, S. dysenteriae and L. monocytogenes.* Results in Table 3, highlighted the remarkable antibacterial activity of acetone root extract against all the tested bacteria with maximum Z.O.I. (25.33 mm) against all the four bacteria viz., *S. aureus, E. coli, S. dysenteriae* and *L. monocytogenes* and minimum Z.O.I. (17.33 mm) against *E. coli.* Methanol root extract also showed remarkable antibacterial activity with maximum Z.O.I. (25.67 mm) against *S. aureus* and *E. coli.*

Screening of antioxidant activity of different extracts of *R. australe*

Results shown in Table 4 is exhibiting that ascorbic acid (0.1 mM in methanol, used as control) has a very good antioxidant potential of IC_{50} of 5.84 µg/mL. showed antioxidant activity of acetone and methanol extracts of different parts (leaf and root) of R. australe. Table 5, showed maximum antioxidant activity (%) in methanol leaf extract with IC_{50} value of 20.37 µg/mL and minimum antioxidant activity (%) in acetone leaf extracts with IC_{50} value of 27.12 µg/mL. Table 6, showed the free radical scavenging activity (%) of acetone (IC_{50} 23.55 µg/mL) and methanol (IC_{50} 25.21 µg/mL) root extracts.

Discussion

Screening of antibacterial activities of different extracts of *R. australe*:

The antibacterial properties of both leaf and root extracts were examined using acetone and methanol at varying concentrations. For the root, the methanol extract demonstrated a slightly higher zone of inhibition (Z.O.I.) of 25.67 mm against *S. aureus* and *E. coli*, compared to the acetone root extract which recorded 25.33 mm and showed equal effectiveness against all four tested bacteria *i.e. S. aureus, E. coli, S. dysenteriae, and L. monocytogenes* at a 100% concentration. Conversely, in the case of the leaf extracts, the acetone leaf extract exhibited a greater Z.O.I. of 19.67 mm against *L. monocytogenes*, while the methanol leaf extract had a Z.O.I. of 15.33 mm against *S. aureus*, both at a 100% concentration.

The antibacterial, insecticidal, and antifungal properties of the crude extracts from *Rheum australe*, along with five other plant species, were examined against six pathogens *i.e. Citrobacter freundii, Escherichia coli, Enterobacter sterogenes, Staphylococcus aureus, Fusarium solani,* and *Aspergillus niger*. The methanol extract of *R. australe* demonstrated antibacterial effects against *S. typhi, S. aureus, P. aeruginosa,* and *C. freundii,* showing the strongest antibacterial activity against *E. coli* and *E. aerogenes* (Hussain *et al.*, 2010). Gupta *et al.*, (2014) examined various extracts of *R. australe* (150 g) using hexane (1000 ml, 7 h), ethyl acetate (1000 ml, 14 h), methanol (1000 ml, 22 h), and finally distilled water (1000 ml, 26 h) through a Soxhlet extractor, revealing significant antimicrobial properties. The methanol extract, following 24 hours of incubation, effectively suppressed the growth of both Gram-positive and Gram-negative bacteria, exhibiting a zone of inhibition (Z.O.I.) ranging from 19 to 23 mm. The ethyl acetate extract was also effective against all bacteria tested, with the exception of *E. cloacae*. The aqueous extract showed antibacterial activity as well, apart from *E. coli* and *S. typhimurium,* while the hexane extract was effective against *E. cloacae* and *E. coli* only.

Screening of antioxidant activities of different extracts of *R. australe*

The free radical scavenging activity (%) of acetone leaf extracts in terms of IC_{50} value was 27.12µg/mL, while for the methanol leaf extract, it was 20.37 µg/mL (IC_{50}). The free radical scavenging activity (%) for acetone root extracts was recorded at 23.55 µg/mL (IC_{50}) and for methanol root extracts, its IC_{50} value was 25.21 µg/mL.

Lin Hu and colleagues (2014) examined the aqueous and methanol extracts of *R. australe* using the DPPH

free radical-scavenging assay. Among these compounds, rheumaustralin exhibited a relatively significant antioxidant activity with an IC50 value of 2.3 µM, which is lower than that of piceatannol (IC50 = 0.14 µmol/L) and higher than that of resveratrol (IC50 = 15.6 µmol/L). Rajkumar *et al*., (2015) investigated the methanol and water extracts from the rhizome of *R. australe*using the DPPH free radical scavenging assay. Both extracts demonstrated a scavenging effect on DPPH radicals that was dependent on their concentration. The methanol extract exhibited a greater ability to scavenge radicals compared to the aqueous extract.

Conclusion

It can be concluded from the present investigation that *R. australe* contained considerable antibacterial and antioxidant potential which can be attributed to the presence of different phytochemicals in their leaves and roots. In case of root, methanol root extracts showed slightly greater (Z.O.I. 25.67 mm), equal against all the four bacteria (*S. aureus, E. coli, S. dysenteriae and L. monocytogenes*) and acetone root extracts showed lesser (Z.O.I. 25.33 mm) equal against *S. aureus* and *E. coli* at highest concentration 100%. In case of leaf, acetone leaf extracts showed greater (Z.O.I. 19.67 mm), against *L. monocytogenes* and methanol leaf extracts showed lesser (Z.O.I. 15.33 mm) against *S. aureus* at highest concentration 100%. DPPH free radical scavenging activity (%) of methanol leaf extract was (IC_{50}= 20.37 µmol/L) and acetone leaf extract was (IC_{50} = 27.12 µmol/L). DPPH free radical scavenging activity (%) of methanol root extract was (IC_{50} = 25.21 µmol/L) and acetone root extract was (IC_{50} = 23.55 µmol/L).

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