Research Article

Antibacterial activity of *Rhus succedanea* Var. *Himalaica*

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Abstract

The antibacterial activity of leaf and root ethanolic extracts as well as crude alkaloids and flavonoids from both the parts of *Rhus succedanea* were carried against bacterial strains viz. *Staphylococcus aureus*, *Clavibacter michiganensis*, *Proteus vulgaris* and *Xanthomonas spp*. The antibacterial activity was carried out on nutrient agar medium using disk diffusion method. The flavonoid fraction of root showed significant antibacterial activity against *Staphylococcus aureus*, *Clavibacter michiganensis*, *Proteus vulgaris* and *Xanthomonas spp* with 24, 25, 15 and 23 mm zone of inhibition respectively; followed by leaf flavonoid fractions with 22, 18, 19 and 20 mm zone of inhibition at 1000µg/ml as compared to reference drug (streptomycin) having 27, 26, 26 and 26 mm at concentration of 0.5 µg/ml respectively. While ethanolic extract and other fractions showed dose dependent activities in order of highest root alkaloids < leaf alkaloids < leaf ethanolic extract and lowest by root ethanolic extract. MIC value confirmed that both alkaloids and flavonoids fraction were more potent than ethanolic extracts of root and leaves. The present study showed that *R. succedanea* have good significant antimicrobial potential against both gram positive and gram negative bacterial strains.

Key Words: *Rhus succedanea*; Antibacterial activity; Leaf and root; Ethanolic extracts; Disk diffusion method; Zone of inhibitions

Introduction

Rhus succedanea var. *himalaica* J. D. Hooker family anacardiaceae is a perennial small sized deciduous tree, grow up to height of 5–9 m tall, known locally by the name Sumac in English, Rakhkal in Pashto and Kakarsingi in Urdu. *Rhus* is an allergic plant due to presence of volatile oil Uroshiol and white latex. It causes a severe dermatitis when an allergic person comes in contact with. *Rhus* has imparipinnately and a highly-branched tape root system with yellow flower forming paniculate inflorescence [1].

Bacterial and fungal infections are responsible for many deaths, each year. The use of antibiotics has revolutionized the treatment of various bacterial and fungal infections. But due to resistance of microbes to antibiotics, it is necessary to assess natural plant resources for developing antimicrobial substances [2]. Plant secondary metabolites like alkaloids, flavonoids, tannins etc have natural antimicrobial properties [3]. Phytochemical natural antimicrobial agents are thought to be environment friendly, easily available and can be used as bio-control agents [4].

Phytoalexins from medicinal plants are naturally defensive compound for all kinds of living organism and helpful in treatment, prevention and control of multidrug resistance strains of bacteria and fungi extracted from medicinal plants [5]. In present research, the *R. succedanea* crude extracts were explored for antibacterial potential against different strains of bacteria.

Materials and methods

Collection and preservation of plant

The fresh root and leaf specimens of *Rhus succedanea* var. *Himalica* were collected from Shalmanay Kotkay, District Shangla, taken to the Department of Botany, University of Peshawar, where identification confirmed by Curator Mr. Ghulam Jelani. The Samples were air dried, mounted on Herbarium sheet and provided Voucher specimen number Bot. Khan, 2007 (PUP) and deposited in the herbarium.

Preparation of leaf and root ethanolic extracts

After pulverization of air dried root and leaf were grind through electric grinder and 1000g powder was soaked in 6000ml of ethanol for 7 days in round bottom flask with continuous shaking the flask every day. The extract was then filtered and the solvent was evaporated through rotary vacuum evaporator and the remainder was

transferred into petridishes and stored in refrigerator.

Total alkaloids fraction

300 ml of 10 percent acetic acids solution was added to the 10g ethanolic extract of root and leaf in a beaker and kept for four hours, covered with aluminum foil. The solution was then concentrated to 1/4th of its original volume on water bath followed by the addition of concentrated NH_4OH . Precipitation occurred, which was filtered through Whatman filter paper, thoroughly washed with dilute ammonium hydroxide and the residues were collected as crude alkaloid [6].

Total flavonoids fraction

10g of each root and leaf extracts were dissolved in 200ml of methanol, containing 80% of aqueous solution, refrigerated for one night. On next day added chloroform to the solution (for glycosides flavonoids) drop after drop and transferred to a beaker, placed on water bath, evaporated to dryness and the remaining extract was the crude flavonoids [3, 7].

The antibacterial activity of leaf and root extracts as well as crude alkaloids and flavonoids fractions from both the parts of *Rhus succedanea* were carried against various strains of bacteria using disk diffusion method Figure 1. [4, 8].

Figure 1. Antibacterial activity of *R. succedanea* against all the tested bacterial strains

Material needed

Bacterial test organism, Nutrient agar, Plant extracts, plant alkaloids and flavonoids, petri dishes, distilled water, Autoclave, Laminar flow hood, Incubator, filter paper, cotton swab, Standard antibiotic (Streptomycin), and micropipettes.

Bacterial strains used; Gram-positive
Staphylococcus aureus, *Clavibacter michiganensis*.

Gram-negative

Proteus vulgaris, *Xanthomonas spp*,

Procedure

Antibacterial activity of the test samples was carried out in research laboratory Agriculture University Peshawar. The Bacterial test organisms were cultured on nutrient broth and incubated for 24 hours before the start of experiments. Then the culture media was prepared by taking 20g of Nutrient agar in 1000ml of distilled water and autoclaved at 137°C for 15 minutes and allowed to cool to 40°C and poured into previously sterilized petri dishes (20ml to each petri dish) inside the laminar flow. Small circular disk of filter papers was transferred to the media and kept at equal distance. The bacterial strains were streaked on media through cotton swab. Then the test

samples were dissolved in ethanol 20mg/ml and applied at 10, 100 and 1000µl/ml dilution on filter paper disks. Ethanol and streptomycin were served as negative and positive control respectively. All the experiments were performed in triplicates. The petri dishes were then incubated at 37°C and antimicrobial effect was observed by zone of inhibition in mm after 24 hours.

Minimum inhibition concentration (MIC)
 MIC was calculated following method of [8, 9]. Extracts that exhibited inhibition at lowest dose 10µg/ml were diluted further by the decreasing serial dilution to get the Minimum Inhibition Concentration.

Results and discussion

Chemist and pharmacist try to discover safe, economic and easily available substances from natural sources [9]. Plant extracts showing antimicrobial activity are a prospective source of innovative natural antimicrobial substances [10]. The antimicrobial activity of *R. succedanea* root and leaf extracts and their isolated crude alkaloid & flavonoid fractions was carried out separately (Figure 1) the results were recorded as Mean and SEM in Table 1 and 2.

Table 1. Antibacterial activities (zone of inhibition in mm) of ethanolic extracts, alkaloids and flavonoids of *R. succedanea* Root against bacterial test organism

S. No.	Bacterial strains	Control	Zone of Inhibition (mm)								
			Root ethanolic extract			Root Alkaloids			Root Flavonoids		
			Concentration µg/ml								
		Streptomycin 0.5 µg/ml	10	100	1000	10	100	1000	10	100	1000
1	<i>Staphylococcus aureus</i>	27±0.5	8±1.16	12±1.04	15±1.5	--	9±1.5	17±1.07	11±0.7	19±2.1	24±3.2
2	<i>Clavibacter michiganensis</i>	26±1.5	--	10±1.8	25±1.5	7±1.6	12±1.04	19±2.1	9±1.5	17±1.07	25±1.5
3	<i>Proteus vulgaris</i>	28±1.04	--	4±0.3	8±1.16	6±0.9	9±1.5	19±2.1	--	8±1.16	15±1.2
4	<i>Xanthomonas spp</i>	26±1.5	3±0.4	6±0.9	10±1.8	10±1.8	15±1.2	19±2.1	14±2.0	20±2.04	23±1.9

Table 2. Antibacterial activity of (zone of inhibition mm) ethanolic extracts, alkaloids and flavonoids of *R. succedanea* against bacterial test organism leaf

S. No.	Bacterial strains	Control	Plant parts								
			Leaf ethanolic extract			Leaf Alkaloids			Leaf Flavonoids		
			Concentration µg/ml								
			10	100	1000	10	100	1000	10	100	1000
1	<i>Staphylococcus aureus</i>	27±0.5	4±0.3	14±2.0	25±1.5	--	17±1.07	20±1.99	--	11±0.7	22±2.01
2	<i>Clavibacter michiganensis</i>	26±1.5	--	6±0.9	9±1.5	--	11±0.7	15±1.5	--	8±1.16	18±1.07
3	<i>Proteus vulgaris</i>	26±1.5	9±1.5	14±2.0	21±2.01	--	10±1.8	22±2.01	4±0.3	8±1.16	19±2.1
4	<i>Xanthomonas spp</i>	26±1.5	--	8±1.16	16±0.89	15±1.5	21±2.00	23±	8±1.16	16±0.26	20±2.04

Roots extracts and its alkaloid and flavonoid fractions

The ethanolic extract of root showed 18, 12, 15mm average zone of growth inhibition against *S. aureus* at doses of 10 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ respectively as compared to the standard control Streptomycin, which showed 27mm average zone of inhibition. While no inhibition was found against *C. michiganensis* and *P. vulgaris* at 10 $\mu\text{g/ml}$. The lowest activity was also recorded at 100 and 1000 $\mu\text{g/ml}$ with mean inhibition of 10, 25 and 4, 8mm respectively compared with control 26 and 28mm respectively. The root extract was least effective against *Xanthomonas spp* (Table 1).

The root alkaloids fraction showed no activity at 10 $\mu\text{g/ml}$ while at 100 and 1000 showed 9 and 17mm mean zone of inhibition. The alkaloids showed highest inhibition against *Xanthomonas spp* with mean 10, 15 and 19 zone of inhibition, while it moderately inhibited the growth of *P. vulgaris* and *C. michiganensis* at same concentrations shown in Table 1. The root flavonoids showed highly significant result against all the tested organisms (Table 1). The average zone of inhibition showed by flavonoids was 11, 19, 24mm and 9, 17, 25mm against *S. aureus* and *C. michiganensis* compared with the control 27 and 26mm respectively. While it inhibited growth of *P. vulgaris* and *Xanthomonas spp* with 9, 17, 25mm and 14, 20, 25mm average

zone of inhibition at the doses of 10, 100 and 1000 $\mu\text{g/ml}$ respectively (Table 1).

Leaf extracts and its alkaloid and flavonoid fractions

The ethanolic extract of leaf showed significant antibacterial activity against *S. aureus* and *P. vulgaris* showing average zone of inhibition of 4, 14, 25mm and 9, 14, 21mm at doses of 10, 100 and 1000 $\mu\text{g/ml}$ respectively comparing with the standard control streptomycin showing 27 and 26mm average zone of inhibition. *C. michiganensis* and *Xanthomonas spp* growth was not inhibited at 10 $\mu\text{g/ml}$ while at 100 and 1000 $\mu\text{g/ml}$ showed lowest inhibition (Table 2).

The crude alkaloid and flavonoid fractions showed highly significant activity against *Xanthomonas spp* (15, 21 and 23mm average zone of inhibition) as compared to control (26mm). Leaf flavonoid showed moderate average growth inhibitory zone 4, 8 and 19mm against *P. vulgaris*, while *C. michiganensis* and *S. aureus* were least inhibited by both alkaloids and flavonoids fractions at all the respective doses (Table 2).

Minimum inhibition concentration (MIC)

MIC value was assessed for those extract that possessed good antibacterial potential and maximum mean inhibition of bacterial growth.

MIC is the lowest antibiotic concentration that inhibits visible growth of bacteria and used as a research tool for determination of *in vitro* activity of new antimicrobial

substances. The MIC values range from 0.5µg/ml, 0.6(µg/ml), 0.7µg/ml, 0.8µg/ml that was exhibited by root and leaf alkaloids and flavonoids which were much similar to the standard drug Streptomycin followed by 1.0µg/ml, 2.0µg/ml and 2.4(µg/ml) exhibited by root and leaf crude ethanolic extracts Table 3. Among all the extracts the leaf flavonoids give good MIC values followed by root flavonoids Table 3.

So it is observed from the above study and MIC determination that all the extracts exhibited have good antimicrobial potential against both gram positive and gram negative bacteria even at very low concentration. It is required to carry out advanced phytochemical work on *R. succedanea* to isolate the precise and specific antibacterial constituents and used as substitutes of synthetic antibiotic drugs.

Similar antibacterial activities were performed by earlier workers and reported results that are strongly in lineage with the present work like [5, 9, 11] also reported that extracts and isolated compounds of *Lawsonia inermis*, *Buddleja salviifolia* *Amaranthus viridis* and *Artemisia*

sieversiana showed highly significant antibacterial effect against various gram positive and gram negative. [12] reported *Berberis baluchistanica*, *Iphiona aucheri*, *Ferula costata* and *Seriphidium quettense* have mild antibacterial effect. Several other researchers like Pendota *et al.* [5], Rauha *et al.* [8], Essawi and Srour. [13], Chaudhary *et al.* [14] and Iqbal *et al.* [15] reported similar results for *Posidonia oceanica*, *Salvia plebeian*, *Salvia cabulica*, *Geranium wallichianum*, *Acacia modesta*, *Colebrookea oppositifolia* and suggested that the plant possess antimicrobial potential due the presence of several phytoconstituents like alkaloids, flavanoides, phenols, tannins, saponins, phlobatannins, sterols and alkaloids. Other Similar works were carried out by Adwan *et al.* [16], Kakar *et al.* [17], Bhalodia *et al.* [18], Hussain *et al.* [19] and Kalase *et al.* [20] which are in analogy with the present work. Our present report on *R. succedanea* plant also shows good antimicrobial potential, therefore, this has prominent perspectives in developing antimicrobial agents through further biological research.

Table 3. Minimum inhibition concentration value of ethanolic extracts, alkaloids and flavonoids of of *R. succedanea* Root and leaf against bacterial test organism

S.No	Bacterial strains	Zone of Inhibition (mm)					
		Root ethanolic extract	Root Alkaloids	Root Flavonoids	Leaf ethanolic extract	Leaf Alkaloids	Leaf Flavonoids
		Minimum Inhibition Concentration value (µg/ml)					
1	<i>Staphylococcus aureus</i>	1(µg/ml)	-----	0.5(µg/ml)	2.4(µg/ml)	-----	0.7(µg/ml)
2	<i>Clavibacter michiganensis</i>	----	0.7(µg/ml)	0.5(µg/ml)	-----	-----	0.6(µg/ml)
3	<i>Proteus vulgaris</i>	-----	0.8(µg/ml)	-----	1.2(µg/ml)	----	0.5(µg/ml)
4	<i>Xanthomonas spp</i>	2.0(µg/ml)	1.2(µg/ml)	0.6(µg/ml)	-----	0.8(µg/ml)	1.0(µg/ml)

Conclusion and recommendations

Our present work concluded that the *R. succedanea* showed highly significant antibacterial activity against both gram negative and gram positive strains of bacteria. That revealed the presence of

highly active antimicrobial phytoconstituents. So it is suggested and recommended that further advance and narrow work should be done on the present plant and the specific responsible agent should be identified, extracted and isolated,

which will a reliable, economic and safe source of antibiotics instead of synthetic haphazard drugs.

Author's contributions

Conceived and designed the experiments: M Ibrar, Performed the experiments: SA Khan, Analyzed the data: S Ullah, Contributed reagents/ materials/ analysis tools: Barkatullah, Wrote the paper: SA Khan.

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