

***In vitro* Antibacterial Activity of *Vitis vinifera* Leaf Extracts against some Pathogenic Bacterial Strains**

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Abstract: Grape (*Vitis vinifera*) usually grows well in temperate countries. Many study show that consumption of grape and various other parts of the fruit, especially the seeds, provides many health benefits. The purpose of this study was to explore the antimicrobial activity of *Vitis vinifera* leaf extract. Sample consisting of fresh healthy leaves that were tested for their properties to inhibit growth of four species of bacteria, namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. Disc diffusion test method was used to determine the ability of the extract to inhibit the bacteria growth. The inhibition zones against the tested bacteria were ranging from 23.7 to 30 mm. The highest zone of inhibition produced by hot water extract *S. aureus* was (30 mm), *E. faecalis* (28.9 mm), *E. coli* was (28 mm) and *P. aeruginosa* (23.7mm). The extract of *Vitis vinifera* leaf was found to be valuable antibacterial agent. Leaves extract was able to inhibit the growth of *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*, but their inhibitory activity against *P. aeruginosa* was less. This study confirms that *Vitis vinifera* leaf extract has antimicrobial activity that could be beneficial to human health.

Key words: Medicinal Plant • Bacteria • ATCC • *Vitis Vinifera* • KPK

INTRODUCTION

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in foods is increasing due to increase in outbreaks of food borne disease [1]. Currently there is a growing interest to use natural antibacterial compounds, like plant extracts of herbs and spices for the preservation of foods, as these possess a characteristic flavor and sometimes show antioxidant activity as well as antimicrobial activity [2].

Grapes (*Vitis vinifera*) belong to family *Vitaceae* [3, 4]. *Vitis vinifera* is a deciduous woody climber with coiled climbing tendrils and large leaves. It has small, pale, green flowers in the summer followed by bunches of berry fruits that range from green to purple-black [5]. Grapes may contain seeds or can be seedless. There are many reports on the benefits of eating grapes as they are known

to be packed with nutrients such as magnesium, vitamins (A, B1, B2, B6 and C) and possess antioxidants properties. Grapes are effective as anti-aging agents through the effects of resveratrol, a molecule in the skin pulp [3, 4].

In Iran, grape leaves are used in a traditional food (vine leaf dolma) and for treatment of diarrhea and bleeding [6]. Grape leaves with antioxidant activity have been reported to treat chronic venous insufficiency in human and nephrotoxicosis induced by citrine [7, 8]. A number of *in-vivo* and *in-vitro* studies were conducted on the plant material and have revealed that *Vitis vinifera* leaves exert various biological activities including hepatoprotective, spasmolytic, hypoglycemic and vasorelaxant effects [9, 10].

Polyphenols are the most important phytochemicals in grapes that possess many biological activities and health-promoting benefits [11, 12]. The phenolic compounds mainly include anthocyanins, flavanols, stilbenes (resveratrol) and phenolic acids [13, 14].

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Vitis vinifera leaf contain mostly myricetin, ellagic acid, kaempferol, quercetin, gallic acid [15]. The grapes water have effective property in primary stage of cancer [16]. In another study characterized grape water reduces risk of platelet aggregation and atherosclerosis [17]. The grapes water has indication in treatment of hypertension. The consumption of grapes water diminishes Leech can cause haemoptesis, nose bleeding, respiratory protein oxidation human [18]. Moreover, grapes leaf has been used for chronic fatigue syndrome (CFS), diarrhea, heavy menstrual bleeding, uterine bleeding and qcancer sores. It has been also used as a mild laxative for constipation [19,20]. The anti-leishmanial activity of the aqueous and ethanolic extract of *Vitis vinifera* leaves were also determined [21].

Plant polyphenols have been demonstrated potential antibacterial [22,23], antifungal [16, 24] and antiviral [25,26] activities. Phenolic compounds in grapes such as resveratrol displayed potent antifungal activity against the human pathogenic fungi *Candida albicans* at concentrations of 10–20 uL. The notable benefit of Phenolic was no induction of hemolytic activity against human erythrocytes, compared to chemical medicines [16]. It has acted as a practicable antimicrobial agent for salad vegetables unconsciously due to its immediate inhibition against *Salmonella typhimurium* [27]. The extracts of *Vitis vinifera* seed exhibited antimicrobial activity to some pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* [28]. Grapes skin possessed the strongest activity in anti-*Helicobacter pylori*, followed by grape synergy (skin and seed) and seed. The increase order of the antimicrobial activity was flesh, whole fruit grapes extracts, fermented pomace, skin and seed [29]. It was also proven that the aqueous extract of *Vitis vinifera* leaves shows antibacterial activity against *Vibrio alginolyticus* [30].

The present study was conducted to investigate the antibacterial activity of *Vitis vinifera* leaf extracts against Gram positive ATCC (American type cell Culture) bacteria *Staphylococcus aureus* ATCC®6538, *Enterococcus faecalis* ATCC®2365 and Gram negative bacteria *Escherichia coli* ATCC®25922 and *Pseudomonas aeruginosa* ATCC®2435.

MATERIALS AND METHODS

Lab Work: Lab work was conducted at Microbiology Research Laboratory, Hazara University Mansehra, Khyber Paktunkhwa (KPK), Pakistan.

Plant Materials: The healthy leaves of *Vitis vinifera* were collected and washed with tap water. After cutting leaves into small pieces, they were air dried at room temperature for 14 days. The dried leaves were crushed into a fine powder by blender machine.

Hot Water Extraction: The method of Adebayo and Ishola [31] was used for extraction. About 5g of the powdered leaf was soaked in 50 ml water in 250 ml sterile flask and placed in incubator for 2 hours at 37°C. The extract was filtered through a muslin cloth and then centrifuged at 4400 rpm for 7 minutes. The supernatant was collected and the pellet was discarded. These steps were repeated three times. The coming supernatant was considered as 100% concentration of extract. The hot water extracts were evaporated to dryness using a rotary evaporator (Stuart, Barloworld and Model RE 300). Their crude extracts were evaporated in a water bath to give gummy solid residue. The obtained crude extracts were 0.54 g.

Media Preparation

Nutrient Agar: Nutrient Agar was enrichment medium for the growth of microorganisms. The Medium was prepared by adding 28g of dehydrated powder using electrical balance into 1 liter of distilled water. PH was adjusted by electrical pH meter at 7.4 and was boiled to dissolve completely.

Media Sterilization: All Media were sterilized by using automatic autoclave (SANYO) at 121°C for 15 minutes.

Media Pouring and Drying: Media was poured in pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

Tested Microorganisms: The *in-vitro* activity of the extracts was assayed against the bacterial strains. All the ATCC (MicroBioLogics) against Gram positive bacteria *S. aureus* ATCC®6538 and *E. faecalis* ATCC®2365 Gram negative bacteria *E. coli* ATCC®25922 and *P. aeruginosa* ATCC® that were provided by Department of Microbiology, Hazara University, Mansehra. Strains were maintained on Nutrient Agar Tubes at 4 °C. The antibiotic efficacy of the plant extracts was evaluated against given strains.

Standardization of Inoculums: After the incubation time, single selective colony of each bacterium from their respective selective agar medium was inoculated into 5ml NB and incubated for 4-6 hours at 37°C in incubator (NAPCO). Sulphuric acid (H₂SO₄) 1% v/v solution was prepared by adding 1 ml of concentrated Sulphuric acid to 99 ml of distilled water. 1.175% w/v solution of barium chloride was also prepared by dissolving 2.35 g of dehydrated barium chloride in 200 ml of distilled water. McFarland Standard No. 0.5 was prepared by mixing 0.05 ml of 1.175% w/v of barium chloride with 9.95 ml of 1% v/v sulphuric acid.

Nutrient agar plates were prepared and pure bacterial cultures were swabbed in these plates and incubated for 37 °C for 24h. With the help of sterile wire loop, three to four well isolated colonies from each plate were transferred to test tubes containing fresh nutrient broth to make bacterial suspension. These test tubes were incubated at 37°C for six hours. Turbidity of these suspensions was adjusted by using nutrient broth to McFarland Standard No. 0.5 by visually comparing the turbidity of bacterial suspension with McFarland standard.

Inoculation of Tested Organisms: Bacterial suspensions (100µl) equivalent to McFarland No. 0.5 were aseptically introduced and spread using pre-sterilized cotton swabs on surface of MHA plates.

Disk Diffusion Method: The disc diffusion method was used to determine the growth inhibition of bacteria by plant extracts [32]. In the disc diffusion method, the filter paper discs were placed aseptically over the bacterial culture on nutrients agar plates and incubated at 37°C for 24 Hrs. After inoculation for 24 Hrs the inhibition zones around the discs were measured by Digital Vernier Caliper (Mitutoyo). The experiment replicated three times to confirm the reproducible results.

Evaluation of Antimicrobial Activity: Antimicrobial activity of *Vitisvinifera* leaf extract was tested using agar disk diffusion method. With the help of sterile micropipette tips *Vitis vinifera* leaf extract (hot water) 3mg/0.1mL were poured into the disk. The plates were incubated at 37°C for 24 Hrs. After incubation, the diameter of the resulting zone of inhibition was measured with the help of Digital Vernier Caliper (Mitutoyo) and the average values were recorded. Each antimicrobial assay was performed three times and means values were reported.

Data Analysis: All data were measured average value of three replicates and standard error (±). Results were subjected to Microsoft excel 2007.

RESULTS

In the present study, the antimicrobial activity of the hot water extracts against two Gram negative and two Gram positive bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones.

Antibacterial Activity: The extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains. Results of the antimicrobial activity obtained using the disk diffusion assay is summarized in Table 1 and 2. Table 1 shows that hot water extract of *Vitis vinifera* leaf make a zone of inhibition on Gram negative bacterial strains including *E. coli* (28 mm) and *P. aeruginosa* (23.7 mm) were determined. Table 2 shows that hot water extract of *Vitis vinifera* leaf make a zone of inhibition on Gram positive *S. aureus* (30 mm) and *E. faecalis* (28.9). Figure 1, Figure 2, Figure 3 and Figure 4 show antimicrobial activity of hot water extract of *Vitis vinifera* against *E. faecalis*, *E. coli*, *P. aeruginosa* and *S. aureus* respectively.

Figure 1-4 show antimicrobial activity of *Vitis vinifera* leaf extract against *E. faecalis*, *E. coli*, *P. aeruginosa* and *S. aureus*. (H.W; Hot water, E; Ethanol, C; control).

DISCUSSION

In this work, we explored antimicrobial activity of *Vitis vinifera* that are grown locally since most reports documented previously were mainly based on grapes grown in temperate climate. The antimicrobial activities of *Vitis vinifera* is due to the presence of alkaloids and flavonoids. The leaves contain flavonoids such as anthocyanins and catechins [33]. In most of the previous researches, extracts of the various parts of the grapes were used, to screen for their antimicrobial activities prospective against all selected pathogens bacterial strains.

The current study suggests that the hot water leaf extract of *Vitis vinifera* has a board spectrum of antimicrobial activity, although the degree of susceptibility could different between different microorganisms. The antimicrobial activity found in this present shown study may be attributed to the presence of

Table 1: Activity of hot water extract of *Vitis vinifera* leaf against Gram negative bacterial strains

Bacterial strains	Zone of inhibition on bacterial strain of <i>Vitis vinifera</i> extract
	Disk diffusion method
<i>E. coli</i>	28 mm
<i>P. aeruginosa</i>	23.7 mm

Table 2: Activity of hot water extract of *Vitis vinifera* leaf against Gram positive bacterial strains

Bacterial strain	Zone of inhibition on bacterial strain of <i>Vitis vinifera</i> leaf extract
	Disk diffusion method
<i>E. faecalis</i>	28.9mm
<i>S. aureus</i>	30 mm

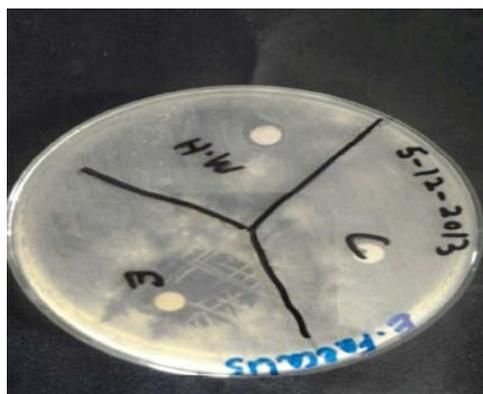


Fig. 1: Antimicrobial activity of *Vitis vinifera* against *E. faecalis*

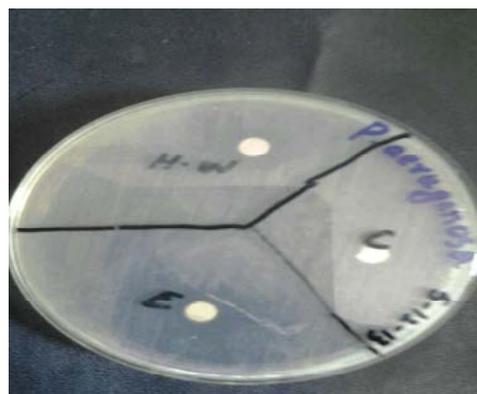


Fig. 3: Antimicrobial activity of *Vitis vinifera* against *P. aeruginosa*



Fig. 2: Antimicrobial activity of *Vitis vinifera* against *E. coli*



Fig. 4: Antimicrobial activity of *Vitis vinifera* against *S. aureus*

secondary metabolites either individually or in combination of various types of chemical composition present in the plant material.

Vitis vinifera leaf fractions were screened for their antibacterial activities against both standard and isolated strains of *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*. The effects of the fractions were compared to those of the control agents (Distal water). They displayed a little more antibacterial activity against *S. aureus*, *E. coli*

and *E. faecalis* bacteria than *P. aeruginosa* bacteria. Rhodes *et al* [34] reported that acidic pH of grape juice of *V. vinifera* was bactericidal and inhibitory to *Listeria monocytogenes* but not against *E. coli* and *S. aureus*. In contrast to their findings, we found that the freshly blended leaf extract from *V. vinifera* has inhibitory effect to *E. coli*, *S. aureus*. As in fresh juice, It is worth noted that the inhibitory extract of grape seed was more effective against Gram-positive than Gram negative [3].

But in our present research zones of inhibition of Gram-negative such as *P. aeruginosa* and *E.coli* are significant. The difference in the result was perhaps attributed to the nature of the test samples whereby in this work, the hot water leaf extract was used while methanolic- and ethanolic-seed extracts was used in their study.

CONCLUSION

From this study, it was concluded that the leaf extract of *Vitis vinifera* grown in the swat valley Pakistan have inhibitory activity against the tested Gram-negative bacteria, *E. coli* and *P. aeruginosa* and Gram-positive bacteria, *S. aureus* and *E. faecalis*. This study gives the technique for further mediation and explores to find out the dynamic compounds accountable for the plant biological activity with the necessary least inhibitory concentration. Secondary research would gather to detect the precise system of action by which extracts employ their antimicrobial effect to diagnose which can be used in drug improvement for innocuous health care

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