

## Antibacterial Activity of Black Grape Leaf (*Vitis vinifera* L.) Extracts Against *Staphylococcus aureus* in-Vitro

<sup>1</sup>Atheer A. Khashan, <sup>2</sup>A.H. Chyad, <sup>3</sup>S.R. Sarhan and <sup>2</sup>O.M.S. Ibrahim

<sup>1</sup>College of Pharmacy, University of Anbar, Ramadi, Iraq

<sup>2</sup>College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

<sup>3</sup>College of Veterinary Medicine, University of Wasit, Kut, Iraq

**Abstract:** The studies turned into meant to investigate inhibitory impact of alcoholic leaf extract of black grape (*Vitis vinifera*) on increase of *Staph. aureus* that has been isolated from skin infections in vitro in which it became drawn *Vitis vinifera*, the usage of ethanol 95% where in the place where the share of extraction of 50% of the weight of dry powder has attended a attention gradient of the alcoholic extract (10-100 mg/mL) had been chosen effective by agar diffusion method and use of micro organism *Staphylococcus aureus* in comparison with ethylene glycol wherein the consequences verified that the diameters of the inhibition of progress of bacterial growth raises with the attention of alcoholic extract concentrations have been 10-20mg/mL low efficiency and concentrations 40-60 mg mL medium performance even as concentrations 80-100 mg/mL changed into pretty powerful and influential in opposition to the increase of micro organism of *Staphylococcus aureus*".

**Key words:** *Vitis vinifera* L, *Staphylococcus aureus*, performance, organism, Staphylococcus

---

### INTRODUCTION

"Microbial interest is an essential technique of disintegration of numerous sustenance's and it's far regularly in rate of the reduction of cost and nicely-being. fear over pathogenic and waste microorganisms in sustenance's is increasing due to growth in episodes of nourishment borne contamination (Tauxe, 1997). As of now you may find a growing enthusiasm to make use of normal antibacterial mixes much like plant concentrates of herbs and flavors for the safeguarding of nourishment as those have a hallmark flavor and now a days after this and then display help movement and additionally anti-bacterial action" (Smid and Gorris, 1999).

Black grapes (*Vitis vinifera*) have a place with family Vitaceae (Jayaprakasha *et al.*, 2003). *Vitis vinifera* is a perishable woody climber with curly climbing rings and extensive takes off. It has little; Pale, green blossoms in the late spring time took after by groupings of berry natural products that range from environmentally friendly to purple-dark (Davis, 1997). Grapes some time contain seeds or can be seedless. Right now there are numerous studies on the great things about eating grapes as they are known to be stuffed with supplements for example, magnesium, vitamins supplements (A, B1, B2, B6 and C) and have cancer prevention agent's properties. Grapes are powerful as against maturing specialists through the impacts of resveratrol an atom in the skin mash (Orallo, 2008).

Inside Iraq, grape leaves are utilized as a part of customary nourishment as well as for treatment of loose

bowels and draining (Zargari, 1993). Grape leaves with cancer prevention agent have been accounted for to treat unending venous in adequacy in human and nephrotoxicosis prompted by citrine (Bilgrami and Jeswal, 1993; Kiesewetter *et al.*, 2000). Various in-vivo and in-vitro studies were led on the plant material and also have uncovered that *Vitis vinifera* Leaves apply different natural exercises including hepatoprotective, spasmolytic, hypoglycemic and vasorelaxant impacts (Montvale, 2002; Orhan *et al.*, 2006).

Polyphenols will be the most critical phytochemicals in grapes which have numerous natural exercises and wellbeing advancing advantages (Shrikhande, 2000; Silva *et al.*, 1991). The phenolic combines for the most part incorporate anthocyanins, flavanols, stilbenes (resveratrol) and phenolic acids (Dopico-Garcia *et al.*, 2008; Spacil *et al.*, 2008).

Plant polyphenols had been proven viable antibacterial (Garcia-Alonso *et al.*, 2006), antifungal (Bruno and Sparapano, 2007) and antiviral (De Bruyne *et al.*, 1999) activities. Phenolic compounds in grapes along with resveratrol proven mighty anti-fungal hobby towards the human pathogenic fungi *Candida albicans* at concentrations of 10-20 uL. The specific great benefit of phenolic changed into now not induction of hemolytic interest in opposition to human erythrocytes in contrast to chemical drugs" (Jung *et al.*, 2005).

"They've acted as an abettor antimicrobial agent for veggies unconsciously because of the instant inhibition towards *Salmonella typhimurium* (Karapinar and Sengun, 2007). The extracts of *Vitis vinifera* seed confirmed

antimicrobial pastime to a few pathogens along with *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Papadopoulou *et al.*, 2005). Grapes possessed the maximum effective hobby in *anti-Helicobacter pylori*, followed via. grape synergy and seed. The rise order of the antimicrobial interest was pores and skin, complete fruit grapes components, fermented pumice, pores and skin and seed (Brown *et al.*, 2009). It ends up additionally tested that the aqueous extract of *Vitis vinifera* Leaves indicates antiseptic activity against *Vibrio alginolyticus* and *Staphylococcus aureus* (Mansour *et al.* 2011). Typically, the existing look at became carried out to investigate the antibacterial hobby of *Vitis vinifera* leaf extracts against *Staphylococcus aureus*".

## MATERIALS AND METHODS

**Culture media:** Were ready in line with the producing companie's instructions and were sterilized in autoclave at 121°C under pressure of 12-15 PSI were incubated at 37°C for 24 h used for culture and diagnosis of bacteria's used in this analysis (Spacil *et al.*, 2008).

**Preparation of plant:** *Vitis vinifera* L was determined as a test herb. Fresh leaves of *Vitis vinifera* L. were accumulated from Baghdad. These leaves were determined at Ministry of Agriculture State Plank for Seed Testing and Certification (S. B. S. T. C.) all leaves were air-dried. Preparing of plant extract.

Dry leaves of the herb were mechanically ground. Leaves powder was extracted with ethanol. Aliquots of get were rinsed for 24 h at room temperatures. The extracts were purified by using Whatman filter paper number 1 and the filtrates were evaporated in incubator at 30°C. (Betty *et al.*, 2007). The resultant focuses were stored in the refrigerator until used.

**Extraction methods:** About 50 g of *Vitis vinifera* L. pieces were smashed in awarding blender for 1 min, then soaked in 450 mL ethanol 95%. It was naturally taken for 3 months at room temperature, the combination was separated in test tubes by centrifugation 3000 rpm, the filtrate was dried in oven 37°C for 24 h. The final product was stored in freezer at (-20) C (Krell, 1996).

**Culture preparation:** Bacteria's were activated by re-culturing on nutrient agar and kept in the incubator for 24 h at 37 C, then used in sterilize tubes containing heart infusion broth, then put in the incubator for 24-72 h at 37C. Total bacterial count number was estimated by using spectrophotometer, the percentages of light transmittance were 27% at a wave duration of 580 nm while the light transmittance was 100% for nutrient broth used to prepare the bacteria (Jassim, 2003).

**Preparaton of standard dilutions of *Vitis vinifera* L.:** The dilution was made by using ethylene glycol which is inert solvent against microorganism (Charles *et al.*, 1969) and by using serial concentrations from 10-100 mg of the extract, then decrease it with ethylene glycol and the amount was completed to 2 mL to get the final concentrations from 1-10%.

***Vitis vinifera* L. extract activity test well diffusion method screening:** Screening of the anti-bacterial activity was performed by well diffusion approach (Saeed and Tariq, 2005). The Mueller-Hinton agar plates were seeded with 0.1 mL of the standardized inoculums of bacteria. The inoculums were spread evenly over dish with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37C for 20 min. A standard crack edge of 9 mm dimension utilized to cut standard wells on the surface of the plates and 0.1 mL of each concentration was presented in the well with ethylene glycol as a control. The inoculated discs were incubated at 37C for 24 h and zone of inhibited diameter was measured to using nearest millimeter (mm).

## RESULTS AND DISCUSSION

**Identification of bacteria:** The bacteria's grow well on mannitol salt aga. Microscopic exam. Gram-positive, spherical in form. Biochemical tests were confirmed the identification of Staph. aureus, catalase and gelatinase +ve, oxidase (-ve), blood agar (B-hemolysis and production of local golden pigment ).

**The inhibitory a result of *Vitis vinifera* L. extract:** The level of sensitivity of the previously pointed out bacteria steadily increased with the increment of attentiveness of extract. The region of the inhibition was 10 mm was written for the concentration of 10 mg/mL and 25 mm was for the concentration 100 mg/mL. The concentrations 10-20 mg/mL were rather low active in protecting against the growth of *Staph. aureus*, the concentrations 40-60 mg/mL were average active while the concentrations 80-100 mg/mL were highly effective compared to ethylene glycol as a control. Table 1 and Fig. 1 shows, there was a proportionate relation between the concentrations of *Vitis vinifera* extract and the diameters of inhibition zones of the expansion of *Staph. aureus*.

Table 1: In vitro inhibitory effect of different concentration of *Vitis vinifera* extract on the growth of *Staph. aureus* measured by dimension of zone of inhibited (mm)

<i>Visits vinifera</i> extract	Con. (mg/mL) Zone of inhibition (mm)
25	100
22	80
18	60
15	40
13	20
9	10

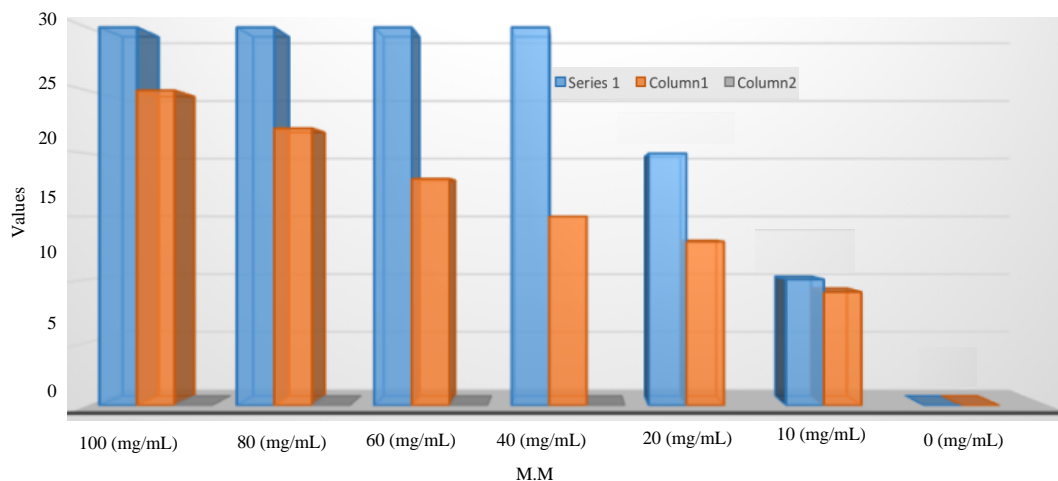


Fig. 1: The *Vitis vinifera* extract a vast spectrum of antimicrobial hobby

In this study, the *Vitis vinifera* L. extract possessed anti-bacterial effect on *Staph. aureus* and the sensitivity of the bacteria was gradually increased with the increasing of extract concentrations Table 1 Bacterial drug resistance is a world problem a high number of bacterial species have become resistant to anti-bacterial drugs (Hebash *et al.*, 1991). Hence, there is a need to evaluate the efficiency of plant chemicals relating to with the growth of bacteria by extracts of plants to be used. Ethanolic extract of *Vitis vinifera* L. extract demonstrated antibacterial activity against *S. aureus*. This effect was agreement with (Kong *et al.*, 2003) who revealed that gram negative microscopic organisms are more safe than gram positive microorganisms to the essential oils which have anti-bacterial activity Nweze and his coworkers (Garcia-Alonso *et al.*, 2006). More or less similar finding were reported in works of other researchers who examined the antimicrobial process of *Vitis vinifera* L. get against *S. aureus* De Bruyne *et al.*, 1999).

The inhibitory effect of *Vitis vinifera* L. on the growth of *Staph. aureus* in this study is due to the important (phenols, flavonoid and tannins) compound in the *Vitis vinifera* L. extract, on the other hand there are different species of the *Vitis vinifera*, plant which have almost the same antibacterial result particularly against *S. aureus* species with comparatively some qualitative and quantitative distinctions in activity which could be attributed to comparative distinctions in the essential chemical constituent of the certain plant species related to many cultivation environmental (temperature and humidity) specialized (time of collection, process of extraction section of the herb used, etc.) or even physiological (growth period, etc.) factors (Chavez *et al.*, 2006; Karapinar and Sengun, 2007; Papadopoulou *et al.*, 2005).

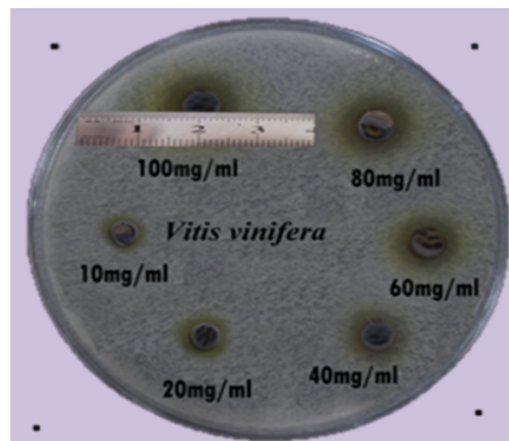


Fig. 2: The inhibitory effect of different concentration of *Vitis vinifera* extract on the progress of *Staph. aureus*

Additionally, the leaves comprise flavonoids consisting of anthocyanins and catechins (Karapinar and Sengun, 2007). In most of the previous researches, extracts of the numerous components of the grapes were used to display screen for his or her antimicrobial activities potential in opposition to all selected pathogens bacterial traces.

The current study suggests that alcoholic extract of leaf *Vitis vinifera* has a vast spectrum of antimicrobial hobby, even though the degree of susceptibility may want to exclusive among specific microorganisms. The antimicrobial hobby determined on this present shown have a look at can be attributed to the presence of secondary metabolites either personally or in mixture of various kinds of chemical composition present within the plant material Fig. 2.

## CONCLUSION

“Using this study it was assumed that the leaf put emphasis of *Vitis vinifera* developed in Iraq have inhibitory activity against the examined Gram-positive microorganisms, *S. aureus*. This study gives the strategy for further involvement and investigates to have the dynamic mixes (in charge of) the rose natural action with the essential tiniest inhibitory fixation. Optional search would assemble to identify the exact arrangement of activity by which takes away utilize their antimicrobial result to diagnose that can be used in drug improvement for innocuous medical”.

## REFERENCES

- Betty, A.F., D.F. Sahm and A.S. Weissfeld, 2007. Bailey and Scotts Diagnostic Microbiology. 12th Edn., Elsevier Mosby, Maryland, USA., ISBN:9780808923640, Pages: 1031.
- Bilgrami, K.S. and P. Jeswal, 1993. Control of citrinin caused nephrotoxicosis through aqueous leaf extract of *Vitis vinifera* L. mercurious corrosivus and cortisone. Indian J. Exp. Biol., 31: 482-484.
- Brown, J.C., G. Huang, V. Haley-Zitlin and X. Jiang, 2009. Antibacterial effects of grape extracts on *Helicobacter pylori*. Applied Environ. Microbiol., 75: 848-852.
- Bruno, G. and L. Sparapano, 2007. Effects of three esca-associated fungi on *Vitis vinifera* L.: V. Changes in the chemical and biological profile of xylem sap from diseased cv. Sangiovese vines. Physiol. Mol. Plant Pathol., 71: 210-229.
- Charles, O.W., G. Ole and F.D. Robert, 1969. Textbook of Organic Medicinal and Pharmaceutical Chemistry. 5th Edn., J. B. Lippincott & Co., Philadelphia, USA.,
- Chavez, J.H., P.C. Leal, R.A. Yunes, R.J. Nunes and C.R. Barardi *et al.*, 2006. Evaluation of antiviral activity of phenolic compounds and derivatives against rabies virus. Vet. Microbiol., 116: 53-59.
- Davis, P.H., 1997. Flora of Turkey and Eas Aegean Islands. Food Res. Intl., 3: 117-122.
- De Bruyne, T., L. Pieters, M. Witvrouw, E. De Clercq and V.D. Berghe *et al.*, 1999. Biological evaluation of proanthocyanidin dimers and related polyphenols. J. Natur. Prod., 62: 954-958.
- Dopico-Garcia, M.S., A. Figue, L. Guerra, J.M. Afonso and O. Pereira *et al.*, 2008. Principal components of phenolics to characterize red Vinho Verde grapes: Anthocyanins or non-coloured compounds?. Talanta, 75: 1190-1202.
- Garcia-Alonso, J., G. Ros, M.L. Vidal-Guevara and M.J. Periago, 2006. Acute intake of phenolic-rich juice improves antioxidant status in healthy subjects. Nutr. Res., 26: 330-339.
- Hebashi, K.A., H.M. Fadel and M. Soliman, 1991. Volatile components of grape leaves. Med. J. Islamic World Acad. Sci., 4: 26-28.
- Jassim, S.J., 2003. Effect of *Haloxylon articulatum* extract on some experimental bacterial and fungal skin infection in dogs. MSc Thesis, Pharmacology and Toxicology Department, College of Veterinary Medicine, University of Baghdad, Iraq.
- Jayaprakasha, G.K., T. Selvi and K.K. Sakariah, 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Res. Int., 36: 117-122.
- Jung, H.J., I.A. Hwang, W.S. Sung, H. Kang and B.S. Kang *et al.*, 2005. Fungicidal effect of resveratrol on human infectious fungi. Arch. Pharmacol. Res., 28: 557-560.
- Karapinar, M. and I.Y. Sengun, 2007. Antimicrobial effect of Koruk (unripe grape-*Vitis vinifera*) juice against *Salmonella typhimurium* on salad vegetables. Food Control, 18: 702-706.
- Kiesewetter, H., J. Koscielny, U. Kalus, J.M. Vix and H. Peil *et al.*, 2000. Efficacy of orally administered extract of red vine leaf as 195 (folia *Vitis viniferae*) in chronic venous insufficiency (stages I-II). Arzneimittelforschung, 50: 109-117.
- Kong, J.M., L.S. Chia, N.K. Goh, T.F. Chia and R. Brouillard, 2003. Analysis and biological activities of anthocyanins. Photochemistry, 64: 923-933.
- Krell, R., 1996. Value-Added Products from Beekeeping. Food and Agriculture Organization, Rome, Italy, Pages: 412.
- Mansour, R., R.L. Ayed, S. Hammam, Z. Mighri and A. Bakhrouf *et al.*, 2011. Antibacterial activities of *Vitis vinifera*. J. Med. Plants Natur. Prod., 26: 126-132.
- Montvale, N.J., 2002. PDR for herbal medicines. Med. Econ., 1: 362-363.
- Orallo, F., 2008. Trans-resveratrol: A magical elixir of eternal youth?. Current Med. Chem., 15: 1887-1898.
- Orhan, N., M. Aslan, D.D. Orhan, F. Ergun and E. Yesilada, 2006. *In-vivo* assessment of antidiabetic and antioxidant activities of grapevine leaves (*Vitis vinifera*) in diabetic rats. J. Ethnopharmacol., 108: 280-286.
- Papadopoulou, C., K. Soulti and I.G. Roussis, 2005. Potential antimicrobial activity of red and white wine phenolic extracts against strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Food Technol. Biotechnol., 43: 41-46.

- Papadopoulou, C., K. Soulti and I.G. Roussis, 2005. Potential antimicrobial activity of red and white wine phenolic extracts against strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *Food Technol. Biotechnol.*, 43: 41-46.
- Saeed, S. and P. Tariq, 2005. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pak. J. Bot.*, 37: 997-1001.
- Shrikhande, A.J., 2000. Wine by-products with health benefits. *Food Res. Intl.*, 33: 469-474.
- Silva, J.M., J. Rigaud, V. Cheynier, A. Chemina and M. Moutounet, 1991. Procyanidin dimmers and trimers from grape seeds. *Phytochemistry*, 30: 1259-1264.
- Smid, E.J. and L.G. Gorris, 1999. Natural Antimicrobials for Food Preservation. In: *Handbook of Food Preservation*, Rahman, M.S. (Ed.). Marcel Dekker, New York, USA., pp: 285-308.
- Spacil, Z., L. Novakova and P. Solich, 2008. Analysis of phenolic compounds by high performance liquid chromatography and ultra performance liquid chromatography. *Talanta*, 76: 189-199.
- Tauxe, R.V., 1997. Emerging foodborne diseases: An evolving public health challenge. *Emerg. Infect. Dis.*, 3: 425-434.
- Zargari, A., 1993. *Medicinal Plants*. 4th Edn., Tehran University Publications, Tehran.