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Biochemistry

Tetrameric Stilbenoid-Hopeaphenol, as Related to the Resistance of Saperavi and Rkatsiteli (*Vitis vinifera* L.) toward Bacterial and Fungal Diseases

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Abstract. Stilbenoids are characterized by various biological activities, among which phytoalexin activity is important. We have established the role of these compounds in the interaction between pathogens and Georgian white and red grape varieties. Stilbenoid biomarkers of Saperavi and Rkatsiteli in Georgian viticulture microzones have been identified as indicators of their resistance to bacterial cancer (*Agrobacterium tumefaciens*), gray mold (*Botrytis cinerea*), and powdery mildew (*Uncinula necator*). The phytoalexin activities of the main stress metabolites of Saperavi and Rkatsiteli (*trans*-resveratrol, *trans*-ε-viniferin, *cis*- and *trans*-piceid) have been determined. The effects of relatively low concentrations of stress metabolites, pallidol and *cis*-δ-viniferin, on the activity of *Botrytis cinerea* have also been studied. The aim of the present work was to determine the phytoalexin activity of the tetrameric derivative of resveratrol - hopeaphenol - against some bacterial and fungal diseases. The phytoalexin activity of hopeaphenol was determined against the bacterial cancer agent – *Agrobacterium tumefaciens*, the gray mold pathogen – *Botrytis cinerea*, and the powdery mildew pathogen – *Uncinula necator* under *in vitro* (laboratory) conditions. Using a water suspension of 5 mg/100 ml of hopaphenol, the biological effectiveness against gray mold for Saperavi was 91.7%, for Rkatsiteli 83.3%. Against powdery mildew, it was 91.7% for Saperavi and 75% for Rkatsiteli. The inhibition of different *Agrobacterium tumefaciens* strains (weak, medium and strong) was related to the concentration of hopeaphenol. *© 2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: Saperavi, Rkatsiteli, phytoalexins, stilbenoids, hopeaphenol

Introduction

Grape stilbenoids are represented by the dominant *cis*- and *trans*-isomeric forms of resveratrol and its derivatives (Riviere et al., 2012; Pawlus et al., 2012). These biologically active compounds are localized in different parts of the vine: shoots, canes, trunk, cluster stems, grape skin, pulp and seeds. Italian scientists have studied stilbenoids in the shoots of 23 grapevine varieties, which are quantitatively mainly present in the form of resveratrol and ε-viniferine, compared to other stilbenoids of lower concentration. The content of stilbenoids in the shoots of the studied varieties ranged from 2700 mg/kg to 6400 mg/kg. A strong influence of the vine variety on the concentration of stilbenoids was revealed (Noviello et. al., 2022). According to some researchers, the content of stilbenoids in grapevine shoots reaches up to 10 g/kg (Houillé et. al., 2015). Seven grapevine varieties shoots from the Veneto region of Italy were studied for stilbenoid accumulation. The main stilbenoids were *trans*-resveratrol, *trans*-piceatannol, and *trans*-ε-viniferin. The influence of pruning time and vine variety factors was confirmed (De Bona et. al., 2020).

Trans-resveratrol, *trans*-ε-viniferin, and 2 tetrameric stilbenoids – one of which is hopeaphenol, were isolated and identified for the first time from the Georgian wine grape variety Rkatsiteli (Bezhuashvili, 1994). Guebailia H. A., et al. (2006) first identified hopeaphenol in North African wines. Among the diverse biological activities of grapevine stilbenoids, phytoalexin activity against fungal and bacterial diseases is important, which has been confirmed by numerous studies. The most important phytoalexin stilbenoids are: resveratrol (Langcake et al., 1976), pterostilbene (Bavaresco et al., 1997), piceid (Waterhouse et al., 1994), viniferins (Flamini et al., 2016). Adrian et al. (2000) studied the variability of stilbenoids in the berry skin of red (Pinot noir, Gamay) and white (Chardonnay) winegrape varieties infected by *Botrytis cinerea* and treated with UV rays.

The antimicrobial activity of stilbenoids has been established in *in vitro* experiments against the sporulation of *Plasmopara viticola*. The most toxic were found to be: δ-viniferin, ε-viniferin, the tetramers viticin-B and hopeaphenol (Schnee et. al., 2013; Chalal et. al., 2014). Richard et al. (2016) studied the phytoalexin activity of grapevine stilbenoid extract against downy mildew (*Plasmopara viticola*) sporulation under greenhouse and vineyard conditions. Infection of grapevine leaves treated with stilbenoids was suppressed by 83-88% in the greenhouse and to a less extent up to 57% in the vineyard. The optimal concentration of stilbenoid extract for sporulation inhibition was determined to be 100 mg/L.

According to Bezhuashvili et al. (2023, 2024), the positive role of stilbenoids against bacterial cancer and fungal diseases – powdery mildew and gray mold of Georgian white and red grape varieties was demonstrated. As a result of our *in vitro* and *in vivo* studies in some viticultural microzones of Georgia, the stilbenoids were correlated to the resistance of Saperavi (in Mukuzani and Napareuli microzones) and Rkatsiteli (in Tsarapi and Tibaani microzones) to gray mold and powdery mildew. The mentioned compounds were represented by the following main stilbenoids*:* *trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, *cis*-piceid, *trans*-astringin, *cis*-astringin, *trans-ε*-viniferin. *trans*-Resveratrol and *trans-ε*-viniferin were important stress metabolite stilbenoids of vine trunks under Crown gall infection of Saperavi and Rkatsiteli (Bezhuashvili et al., 2024). We continued the experiments in that direction, by focusing on the activity of resveratrol tetramer- hopeaphenol in Saperavi and Rkatsiteli vine trunks infected by *Agrobacterium tumefaciens*, grape skins infected by *Botrytis cinerea* and *Uncinula necator* under *in vitro* conditions.

Materials and Methods

The experimental design included healthy and gray mold-artificially infected grape skins of Saperavi (red) and Rkatsiteli (white) varieties, grown in some areas of Eastern Georgia, as follows: Saperavi in vineyards located in Mukuzani (vines 17-year-old) and Napareuli (vines 40 year old); Rkatsiteli – in vineyards located in Tsarapi (vines 40 year old) and Tibaani (vines 17 year old). Berries and trunks samplings were done at harvest (2024).

Stilbenoids containing fractions were isolated from healthy and infected vine trunk and grape skins by extraction ethylacetate and separation on the sephadex G 50. Hopeaphenol were individually isolated from ethyl acetate extract by column separation.

Stilbenoids were determined by the method of high performance liquid chromatography (HPLC) (Guebailia et al., 2006).

Lab-*in vitro*-experiment. Healthy and infected vine trunks, berries of winegrape varieties (*V. Vinifera* L.) Saperavi and Rkatsiteli were sampled at technological maturity (September, 2024) in the locations described above; the experimental design included the following treatments: 1) berry pre-treatment with 1 mg/100 mL, 3 mg/100 mL and 5 mg/100 ml of hopeaphenol, followed by the infection with *Botrytis cinerea* and *Uncinula necator*; 2) control berries with fungal infection, but without pretreatments. The pre-treatments were done by soaking 12 berries per variety in the previously described solutions (or just water in the case of the control), while fungal infection was done by spraying *Botrytis cinerea* and *Uncinula necator* conidial suspension over the berries placed on damp filter paper inside petri dishes. The fungal inoculum was prepared by recovering conidia from infected berries grown in the field.

We isolated stilbenoids containing fractions from the healthy and artificially infected trunks of Saperavi and Rkatsiteli vines from experimental vineyard. We determined the impact of hopeaphenol on the activity of *Agrobacterium tumefaciens in vitro*. The experiment was carried out in the petri dishes on the food area – Potato Dextrose Agar. As a control variant, a water suspension of a pure culture of *Agrobacterium tumefaciens* (a strong pathogenic strain) was directly applied on the food area. In the experimental variants, pre-treated food area with a water suspension of hopeaphenol was used. Then the pure culture of the bacteria was applied on the food area, closed Petri dishes were placed in a thermostat at 37ºC and the growth and development of bacteria during the incubation period was observed.

Results and Discussion

Physiological and stress metabolic concentrations of hopeaphenol were found to be slightly different in the Saperavi and Rkatsiteli trunks, except for the Tibaani microzone in Rkatsiteli. Hopeaphenol concentration increases under infection of bacterial cancer in Saperavi as compared to Rkatsiteli.

Table 1. Change of concentration of hopeaphenol (g/kg) in vine trunks of healthy and infected vines depending on the variety and the location

Stilbenoid	**Location**							
	Saperavi				**Rkatsiteli**			
	Mukuzani		**Napareuli**		**Tsarapi**		**Tibaani**	
	Health	Infected	Health	Infected	Health	Infected	Health	Infected
Hopeaphenol	0.20	0.31	0.10	0.24	0.12	0.10	0.87	0.27

Table 2. Change of concentration of hopeaphenol (g/kg) in grape skin of healthy and infected by fungal diseases grapes depending on the variety and the location

Stilbenoid	Location							
	Saperavi Gray mold (*Botrytis cinerea*)				Rkatsiteli Gray mold (*Botrytis cinerea)*			
	Mukuzani		Napareuli		Tsarapi		Tibaani	
	Health	Infected	Health	Infected	Health	Infected	Health	Infected
Hopeaphenol	3.25	7.90	3.05	4.33	4.40	5.60	1.25	5.69
	Powdery mildew (*Uncinula necator)*				Powdery mildew (*Uncinula necator)*			
	Health	Infected	Health	Infected	Health	Infected	Health	Infected
	4.59	2.05	4.77	4.21	3.57	2.17	1.42	2.93

Table 3. Effect of hopeaphenol concentration on the activity of pathogenic strains of *Agrobacterium tumefaciens*

Pathogenic strains	Watery suspension of hopeaphenol		
	1mg/100ml	**3mg/100ml**	**5mg/100ml**
Saperavi (Mukuzani/Napareuli)	Bacterial multiplication, %, relative to food area		
Weak	50/55	20/23	0/0
Medium	70/70	35/32	1/1
Strong	90/87	40/42	1/2
Rkatsiteli (Tsarapi/Tibaani)	**1mg/100ml**	**3mg/100ml**	**5mg/100ml**
Weak	45/43	17/20	0/0
Medium	65/68	30/35	1/1
Strong	75/80	37/35	1/2

Hopeaphenol concentration significantly decreases in the Rkatsiteli trunks of the Tibaani microzone (Table 1).

Hopeaphenol was detected in significantly higher concentrations in the skin of Saperavi and Rkatsiteli grapes compared to the vine trunks. Hopeaphenol concentration increased in the skin of experimental grapes infected with gray mold in all microzones. In particular, in the skin of Tibaani grapes, it ranged from 1.25 g/kg to 5.69 g/kg. In the skin of infected grapes from Saperavi in the Mukuzani microzone, the highest concentration of hopeaphenol was 7.90 g/kg. Different variations of hopeaphenol were detected in Saperavi and Rkatsiteli grape skins infected with powdery mildew. Specifically, the concentration of hopeaphenol decreased in all experimentally infected organs. The exception was the infected skin of the Tibaani microzone Rkatsiteli grape, in which hopeaphenol was observed from 1.42 g/kg to 2.93 g/kg (Table 2).

The development and spread of pure and pathogenic strains of *Agrobacterium tumefaciens* isolated in the lab from Saperavi and Rkatsiteli grapevine strains was found to depend on the concentration of hopeaphenol. Specifically, water suspension of hopaephenol at a concentration of 5 mg/100 ml completely inhibits the spread of the weak strain. The moderately pathogenic strain has a prevalence of 1%, while the highly pathogenic strain isolated from the Saperavi strain of the Mukuzani microzone has a prevalence of 1%. The strain from the Napareuli microzone has a prevalence of 2%. A similar result was observed for pathogenic strains isolated from Rkatsiteli- Tsarapi microzone spread by 1% and from Tibaani by 2%. The growth of pathogens of all three strengths in the food area treated with each concentration of the water suspension of hopeaphenol differs slightly depending on the microzones (Table 3).

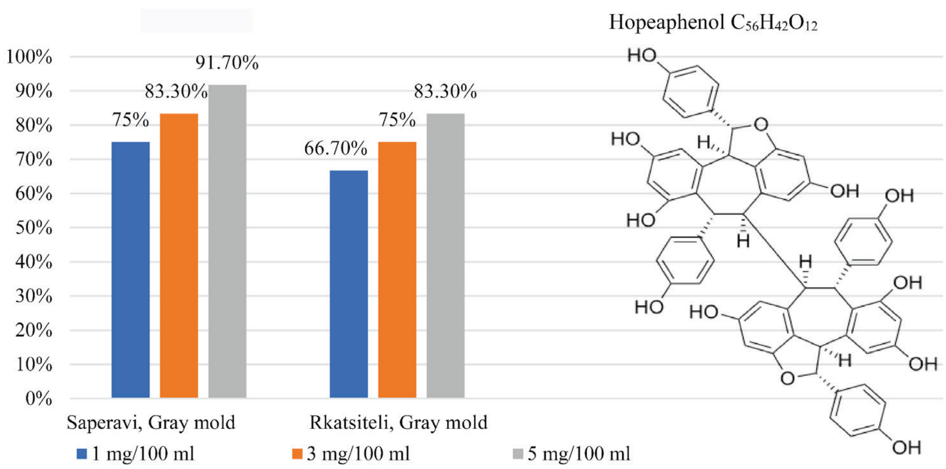

Fig. 1. Biological efficiency (%) of hopeaphenol against Gray mold on Saperavi and Rkatsiteli grape berries.

The biological effectiveness of Saperavi grape berries treated with 5 mg/100 ml of hopeaphenol against *Botrytis cinerea* infection was 91.7%, and for Rkatsiteli it was 83.3%. Infected with *Uncinula necator*, the same biological effectiveness was observed for Saperavi and for Rkatsiteli (75%) (Figs. 1,2).

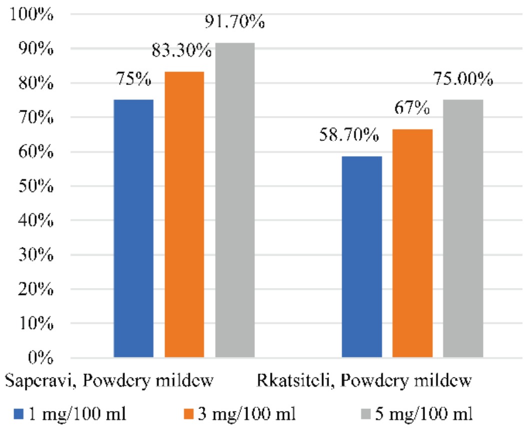

Fig. 2. Biological efficiency (%) of hopeaphenol against Powdery mildew on Saperavi and Rkatsiteli grape berries.

Conclusion

For the first time was established the phytoalexin activity of tetrameric stilbenoid hopeaphenol in Saperavi and Rkatsiteli vine trunks and grape skins against bacterial cancer (*Agrobacterium tumefaciens*), gray mold (*Botrytis cinerea*) and powdery mildew (*Uncinula necator*). The presence of hopeaphenol in the stilbenoid profile of Saperavi (Mukuzani and Napareuli) and Rkatsiteli (Tsarapi and Tibaani) in 4 microzones of viticulture is an indicator of the resistance of these grape varieties against the above bacterial and fungal diseases.

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სტილბენოიდები ხასიათდება მრავალფეროვანი ბიოლოგიური აქტივობით, რომელთა შორის ვაზისთვის მნიშვნელოვანია ფიტოალექსინური აქტივობა. ჩვენ მიერ დადგენილია საქართველოს თეთრი და წითელყურძნიანი საღვინე ვაზის ჯიშების იმუნიტეტის კორელაცია ფიტოალექსინ სტილბენოიდებთან. განსაზღვრულია საქართველოს მევენახეობის მიკროზონებში გავრცელებული საფერავის და რქაწითელის სტილბენოიდური ბიომარკერები, როგორც მათი რეზისტენტობის მაჩვენებელი, ბაქტერიული კიბოს (Agrobacterium tumefaciens), ნაცრისფერი სიდამპლის (Botrytis cinerea) და ნაცრის (Uncinula necator) მიმართ. დადგენილია საფერავის და რქაწითელის ძირითადი სტრეს-მეტაბოლიტი სტილბენოიდების: ტრანს-რესვერატროლის, ტრანს-ε-ვინიფერინის, ცის-და ტრანს-პიცეიდის ფიტოალექსინური აქტივობები. ასევე შესწავლილია პალიდოლის და ცის-δ-ვინიფერინის ზემოქმედება Botrytis cinerea-ს აქტივობაზე. წინამდებარე ნაშრომის მიზანს წარმოადგენდა რეზვერატროლის ტეტრამერული წარმოებულის-ჰოპეაფენოლის ფიტოალექსინური აქტივობის დადგენა ზოგიერთი ბაქტერიული და სოკოვანი დაავადების მიმართ *in vitro* (ლაბორატორიულად). დადგინდა ჰოპეაფენოლის ფიტოალექსინური აქტივობა ბაქტერიული კიბოს გამომწვევი – Agrobacterium tumefaciens-ის, ნაცრისფერი სიდამპლის გამომწვევი – Botrytis cinerea-ს და ნაცრის გამომწვევი – Uncinula necator-ის მიმართ. ჰოპეაფენოლის 5მგ/100მლ კონცენტრაციის წყლიანი სუსპენზიის გამოყენებით, ბიოლოგიურმა ეფექტიანობამ საფერავისთვის ნაცრისფერი სიდამპლის მიმართ შეადგინა 91,7%, რქაწითელისთვის 83,3%, ნაცრის წინააღმდეგ კი, საფერავისთვის – 91,7%, რქაწითელისთვის – 75%. ბაქტერიული კიბოსთვის Agrobacterium tumefaciens-ის სუსტი, საშუალო და ძლიერი შტამების გავრცელების ინჰიბირება მოხდა ჰოპეაფენოლის კონცენტრაციაზე დამოკიდებულებით.

REFERENCES

Adrian, M., Jeandet, P., Douillet-Breuil, A.C., Tesson, L., Bessis, R. (2000). Stilbene content of mature Vitis vinifera berries in response to UV-C elicitation. *J. Agric. Food Chem*., *48,* 6103-6105.

Bavaresco, L., Petegolli, D., Cantu, E., Fregoni, M., Chiusa, G., Trevisan, M. (1997). Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by Botrytis cinerea. *Vitis*, *36*(2), 77-83.

Bezhuashvili, M. (1994). *Vazis da mukhis merqnis ligninis konversiis teoriuli safudzvlebis shemushaveba da migebuli produqtebis gamokenebis gzebis gansazgvra* [Development of theoretical foundations for lignin conversion of vine and oak wood and determination of ways to use the resulting products]. Doctoral Thesis, p. 260. Tbilisi.

Bezhuashvili, M., Bavaresco, L. , Surguladze, M., Kharadze, Sh., Shoshiashvili, G., Darchiashvili, N., Vashakidze, P. (2024). Change of phytoalexin stilbenoids in the vine trunk (*Vitis vinifera* L.) under crown gall infection (Agrobacterium tumefaciens). *Bull. Georg. Natl. Acad. Sci., 18*(2), 133-139.

Bezhuashvili, M., Bavaresco, L., Tskhvedadze, L., Surguladze, M., Shoshiashvili, G., Darchiashvili, N., Vashakidze, P (2023). Phytoalexin stilbenoids of Saperavi and Rkatsiteli (*Vitis vinifera* L.) as biomarkers of resistance to gray mold (*Botrytis cinerea*). *Bull. Georg. Natl. Acad. Sci., 17*(2), 136-141.

Bezhuashvili, M., Bavaresco, L., Surguladze, M., Tskhvedadze, L., Shoshiashvili, G., Darchiashvili, N. and Vashakidze, P. (2024). Change of phytoalexin stilbenoids of *Vitis vinifera* L. cvs. Rkatsiteli and Saperavi under powdery mildew infection in the lab and in the vineyard. 45th World Congress of Vine and Wine, 14-18 October, Dijon, France.

Chalal, M., Klinguer, A., Echairi, A., Meunier, P., Vervandier-Fasseur, D. and Adrian, M. (2014). Antimicrobial activity of resveratrol analogues. *Molecules,* **19**, 7679-7688.

De Bona, G.S., Bonora, F. and Vincenzi, S. (2020). Grapevine canes waste from Veneto region as a new source of stilbenoids content. *Ital. J. Food Sci*., *32,* 310-320.

Flamini, R., Zanzotto, A., de Rosso, M., Lucchetta, G., Vedova, A.D., Bavaresco, L. (2016). Stilbene oligomer phytoalexins in grape as a response to aspergillus carbonarius infection. *Physiological and Molecular Plant Pathology,* 93, 112-118.

Guebailia, H. A., Chira, K., Richard, T., Mabrouk, T. and Furiga, A. (2006). Hopeaphenol: The first resveratrol tetrameric wines from North Africa. *J. Agric. Food Chem.*, *54*, 9559-9564.

Houillé, B., Besseau, S., Courdavault, V., Oudin, A., Glévarec, G., Delanoue, G., Guérin, L., Simkin, A. J., Papon, N., Clastre, M., Giglioli-Guivarc'h, N. and Lanoue, A. (2015). Biosynthetic origin of E-resveratrol accumulation in grape canes during postharvest storage. *J. Agric. Food. Chem*., 63, 1631-1638.

Langcake, P., Pryce, R. J. (1976). The production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.,* 9, 77-86.

Noviello, M., Caputi, A.F., Squeo, G., Michele Paradiso, V., Gambacorta, G., Caponio, F. (2022). Vine shoots as a source of *trans*-resveratrol and ε-viniferin: A study of 23 Italian varieties. *Foods*., *11*(4), 553-565.

Pawlus, A.D., Waffo Téguo, P., Shaver, J. and Mérillon, J.M. (2012). Stilbenoid chemistry from wine and the genus *Vitis*, a review. *J. Int. Sci. Vigne Vin*, **45**, 57-111.

Richard, T., Abdelli-Belhad, A., Vitrac, X., Waffo-Téguo, P., Mérillon, J.-M. (2016). Vitis vinifera canes,a source of stilbenoids against downy mildew. *OENO One, 50*(3), 1178-1185.

Rivière, C., Pawlus, A.D., Mérillon, J.M. (2012). Natural stilbenoids: Distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. *Natural Product Reports, 29*(11), 1317-1333.

Schnee, S., Queiroz, E.F., Voinesco, F., Marcourt, L., Dubuis, P.H., Wolfender, J.L. and Gindro, K. (2013). *Vitis vinifera* canes, a new source of antifungal compounds against *Plasmopara viticola*, *Erysiphe necator*, and *Botrytis cinerea*. *J. Agric. Food Chem., **61**,* 5459-5467.

Waterhouse, A. L; Lamuela Raventos, R. M. (1994). The occurrence of piceid, a stilbene glucoside in grape berries. *Phytochemistr*y, 37, 571-573.

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