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ANTIMICROBIAL PROPERTIES OF WHITE MALAGA GRAPE GROWN IN MALAYSIA

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ABSTRACT

Grapes (Vitis vinefera) are usually produced and grow well in temperate countries. Many reports had shown that consumption of grape and parts of the fruit, especially the seeds, provides many health benefits. The purpose of this study was to explore the antimicrobial properties of grapes that are cultivated in Northern Malaysia. Samples consisting of fresh grape of White Malaga variety, prepared cordials of two varieties Red Opal and White Malaga; and dried grape 'tea' leaves were tested for their properties to inhibit growth of four species of bacteria, namely, Escherichia coli, Klebsiella pneumoniae, Streptococococcus aureus and Streptococococcus epidermidis. Disc diffusion test method was used to determine the ability of the samples to inhibit the growth of the bacteria. An experiment to determine the effect of storage of the samples at 4°C on their antimicrobial activity was also conducted. Results showed that fresh grape juice and both types of cordials were able to inhibit the growth of E. coli and K. pneumoniae, but their inhibitory activity against K. pneumoniae was lost upon refrigeration. By refrigerating the fresh juice, it was observe that the inhibitory activity against S. aureus and S. epidermidis was enhanced. The infused grape 'tea' leaves showed antimicrobial activity to S. epidermidis only after the refrigeration. This study therefore confirms that the White Malaga grape grown in tropical countries like Malaysia has antimicrobial properties that could be beneficial to human health, similar to many other previous reports.

Keywords: Antimicrobial properties, white Malaga grape, Malaysia

INTRODUCTION

Grapes belong to the *Vitaceae* family and under the genus *vitis*. They are believed to be native to Northwest Asia and have been cultivated for centuries in many parts of the Mediterranean, Central Asia and Australia. Although they grow well in temperate regions, grapes can also be grown in the tropical countries like Thailand and Malaysia. They have recently been cultivated on commercial scale in Perlis, Negeri Sembilan and Kedah for local consumptions. Grapes can be eaten fresh, or can be dried as raisins, processed as jams or used in recipes. These grapes are known as table grapes, while grapes for production of wine are called wine grapes. 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 and seeds (Jayaprakasha *et al.*, 2003, Orallo, 2008). Besides longevity, resveratol lowers the cholesterol level by reducing the oxidized lower density lipoprotein cholesterol and can prevent against cardiac heart diseases (Bertelli and Das, 2009). In addition, resveratol may also lower the risk of Alzheimer's disease (Marambaud *et al.*, 2005) and has been reported to possess cancer chemopreventive activity (Gescher, 2008). Resveratol coupled with proanthrocyanidins present in the seed and skin, are two compounds important in reducing the risk of morbidity and mortality of breast cancer (Gehm *et al.*, 1997).

Phenolic compounds from the different parts of the grapes seemed to display different properties that could be beneficial to health. A study in India reported that petroleum-ether extracts of grape seeds inhibited growth of *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Jayaprakasha *et al.*, 2003). In another study, the phenolic extracts from the juice and skin of *V. vinifera* were found to be highly inhibitory to *Listeria monocytogenes* (Rhodes *et al.*, 2006). This antimicrobial property in grapes was anticipated to be useful in the preservation of food products (Baydar *et al.*, 2004). In Turkey, grape leaves are used as folk medicine as ailments for diarrhoea, inflammatory disorder, haemorrhage, wound healing and many other medical disorders (Orhan *et al.*, 2009). The leaves contains flavonoids such as anthocyanins and catechins (Monagas *et al.*, 2006). In most of the previous researches, extracts of the various parts of the grapes were used, to screen for their antimicrobial activities. To most of us, table grapes are usually consumed wholly or taken as juice or cordials, as not many of us take grape extracts as supplements. In this work, we explore on the

antimicrobial properties of table grapes that are grown locally since most reports documented previously were mainly based on grapes grown in temperate climate.

MATERIALS AND METHODS

Preparation of samples

Three varieties of grapes were grown in Beris Lake Vineyard comprising White Malaga, Black Opal and AIC. Freshly picked whole grape fruits from the variety of White Malaga were kept refrigerated at 4°C for one day. Approximately, 500 g of the fruit was thoroughly washed with sterile distilled water and the stalks were removed. The whole fruits were blended without water which produced approximately 220 ml of juice. Preserved red and green grape cordials were used undiluted in this study. In addition, 20 mg of dried grape 'tea' powdered leaves were mixed with 200 ml boiled distilled water and let to infuse at room temperature for 3 min. The pH of the samples was determined prior to test, using a pH meter (Methrohm, Germany).

Bacteria Strains

American Type Culture Collection (ATTC) of bacteria used in the experiment consisted of *Escherichia coli* (ATTC 35218), *Klebsiella aeruginosa* (ATTC 10031), *Staphylococcus aureus* (ATTC 43300) and *Staphylococcus epidermidis* (ATTC 12228). All of the bacteria strains were maintained at -80°C prior to subculture to fresh agar media. These cultures were then incubated overnight at 37°C to be used as the test organisms.

Culture Media

E. coli and *K. pneumoniae* were cultured on MacConkey agar (Oxoid, UK), while sheep blood agar was used to grow *S. aureus* and *S. epidermidis*. For antimicrobial sensitivity test, Muller Hinton (MH; Oxoid, UK), agar was used.

Screening for Antimicrobial Properties

Sterile 6 mm diameter paper discs (Whatman no. 1, UK) were impregnated with 30 µl of the test samples and allowed to dry completely in an oven at 45°C. Discs impregnated with sterile distilled water were used as negative controls. The procedure for the antibiotic susceptibility test was carried out according to the standard method (NCCLS, 2002). In brief, prior to the test, the bacterial colonies on the each of the agar plates were scraped and were diluted with phosphate buffer saline to obtain a suspension equivalent to 0.5 McFarland. Each strain of bacteria were then streaked evenly on the surface of MH agar and allowed to dry at room temperature. Subsequently, the sample and the control discs were placed aseptically on the lawn of bacteria and incubated at 37°C. Following overnight incubation, the zone of inhibition was observed and measured. Each sample was done in duplicates.

Effect of Refrigeration on the Antimicrobial Activities of Grapes

All the test samples were kept at 4°C for 48 h before they were impregnated onto the paper discs and antimicrobial sensitivity test was performed as previously stated.

Results and Discussion

Based on the size of inhibition produced by the grapes and its products as shown in Table I, it was observed that the freshly blended grape juice was able to inhibit *E. coli* and *K. pneumoniae* but not the *Staphylococcus* spp. After storage at 4°C for 48 h, the zone of the inhibition against *E. coli* had slightly increased, and it was also able to inhibit the growth of both species of *Staphylococcus*. Conversely, the antimicrobial property of the fresh grape juice to *K. pneumoniae* was lost upon refrigeration. During the refrigeration, the pH of the fresh grape increased in its acidity. A group of researchers reported that acidic pH of grape juice of *V. vinifera* var. Ribier (black table grape) was bactericidal and inhibitory at pH ≤5.0 to *L. monocytogenes* but not against *E. coli* and *S. aureus* (Rhodes *et al.*, 2006). In contrast to their findings, we found that the acidic pH of the freshly blended juice from *V. vinifera var* Malaga has inhibitory effect to *E. coli*, *S. aureus* and *K. pneumoniae* but as the pH became more acidic during the storage, it loses its inhibitory effect on *K. pneumoniae*. As in fresh juice, similar observations were obtained with both red and green cordials whereby they were able to inhibit the growth of *E. coli* and *K. pneumoniae*. It is worth noted that gram-positive bacteria tested in this work seemed to be more resistant to the inhibitory effect of the grape juice. On the contrary, Jayaprakasha *et al.* (2003) reported that the inhibitory extract of grape seed was more effective against gram-positive than gram-negative

bacteria. The difference in the result was perhaps attributed to the nature of the test samples whereby in this work, the juice from the whole fruit was used while methanolic- and ethanolic-seed extracts was used in their study.

Table I. pH and antimicrobial activities of fresh grape juice, grape cordials and grape 'tea' to four bacteria of medical importance.

	pH		Zone of Inhibition (mm)*							
			E. coli		*K. pneumo*		*S. aureus*		*S. epiderm*	
Storage at 4°C (h)	0	48	0	48	0	48	0	48	0	48
Fresh juice	5.02	4.57	22.8	24.0	27	0	0	32	0	34
Red Grape Cordial	5.39	5.34	24	22	27	0	0	0	0	0
Green Grape Cordial	5.91	5.83	22	19	36.7	0	0	0	0	0
Grape 'tea'	4.22	4.02	0	0	0	0	0	0	0	12
Control (Water)	6.81	6.82	0	0	0	0	0	0	0	0

E. coli = Escherichia coli, K. pneumo = Klebsiella pneumoniae; S. aureus = Staphylococcoccus aureus; Staphylococcus epiderm = S. epidermidis

* An average measurement of duplicates.

They believed that gallic acid in the seed extract is the bioactive compound involved with the antibacterial property. As our juice sample was blended together with the seeds, the gallic acid had played a role in the inhibitory activity. During storage at 4°C, although the grape juice and cordials maintained its inhibitory activity to *E. coli*, its effect on *K. pneumoniae* was lost. We hypothesized that the activity of gallic acid may have deteriorated during storage. This finding agrees to a report that *E. coli* was more sensitive to gallic acid compared to *K. pneumoniae* (Vaquero *et al.*, 2007). This explains the observation that *E. coli* was inhibited whereas *K. pneumoniae* was not sensitive to the gallic acid after storage. Many studies on the benefits of grapes were focussed mainly on the fruit. There are very limited numbers of research which studies on the medicinal benefits of grape leaves. In one report, it was shown that grape leaf extract at low concentration was able to inhibit *L. monocytogenes* (Hara-Kudo *et al.*, 2004). In this study, it was observed that the grape 'tea' which was infused for 3 min did not have any inhibitory effect against the bacteria tested, however, following refrigeration for 48 h, it was able to inhibit *S. epidermidis*. The short duration of infusion could be a factor that did not allow the active compounds to be released. The green 'tea' leaves seemed to reveal its antimicrobial activity against *S. epidermidis* only after storing for 2 days which probably had released the phenolic acid from the leaves. The low pH of the grape 'tea' was due to the presence of tartaric and malic which is presence in grape leaves (Batovska *et al.*, 2008). These two compounds have been shown to have antimicrobial activity to Streptococci (Daglia *et al.*, 2007). Due to this, *S. aureus* was probably less sensitive than *S. epidermidis* to these acids and thus, not inhibited by the grape 'tea'.The poor antimicrobial activity exhibited by the grape 'tea' leaves supported the report of Jayaprakasha *et al* (2003) that grape seed extract was superior to extract of grape leaves.

CONCLUSION

From this *in vitro* study, it can be concluded that the grapes grown in the Northern Malaysia have inhibitory activity to gram-negative bacteria, particularly *E. coli* and *K. pneumoniae* and although refrigeration maintains its inhibitory activity against *E. coli*, it was not effective against *K. pneumoniae*.

REFERENCES

Batovska, D. I., Todorova, I. T., Nedelcheva, D. V., Parushev, S. P., Atanassov, A. I., Hvarleva, T. D., Djakova, G. J., Bankova, V. S.andPopov, S. S. (2008), "Preliminary study on biomarkers for the fungal resistance in Vitis vinifera leaves". J Plant Physiol, 165, pp. 791-795.

Baydar, N. G., Ozkan, G.andSagdic, O. (2004), "Total phenolic contents and antibacterial activities of grape (Vitis vinifera L.) extracts". Food Control, 15**,** pp. 335-339.

Bertelli, A. A. A.andDas, D. K. (2009), "Grapes, Wines, Resveratrol, and Heart Health". Journal of Cardiovascular Pharmacology, 54**,** pp. 468-476.

Daglia, M., Papetti, A., Grisoli, P., Aceti, C., Dacarro, C.andGazzani, G. (2007), "Antibacterial activity of red and white wine against oral streptococci". J Agric Food Chem, 55**,** pp. 5038-5042.

Gehm, B. D., Mcandrews, J. M., Chien, P. Y.andJameson, J. L. (1997), "Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor". Proc Natl Acad Sci U S A, 94**,** pp. 14138-14143.

Gescher, A. J. (2008), "Resveratrol from Red Grapes - Pedestrian Polyphenol or Useful Anticancer Agent?". Planta Medica, 74**,** pp. 1651-1655.

Hara-Kudo, Y., Kobayashi, A., Sugita-Konishi, Y.andKondo, K. (2004), "Antibacterial activity of plants used in cooking for aroma and taste". Journal of Food Protection, 67**,** pp. 2820-2824.

Jayaprakasha, G. K., Selvi, T.andSakariah, K. K. (2003), "Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts". Food Research International, 36**,** pp. 117-122.

Marambaud, P., Zhao, H. T.andDavies, P. (2005), "Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides". Journal of Biological Chemistry, 280**,** pp. 37377-37382.

Monagas, M., Garrido, I., Bartolome, B.andGomez-Cordoves, C. (2006), "Chemical characterization of commercial dietary ingredients from Vitis vinifera L.". Analytica Chimica Acta, 563**,** pp. 401-410.

NCCLS (2002), "Performance standards for antimicrobial disk susceptibility tests. M100-S12", An NCCLS global informational supplement. 22, 60-61.

Orallo, F. (2008), "Trans-resveratrol: A magical elixir of eternal youth?". Current Medicinal Chemistry, 15**,** pp. 1887-1898.

Orhan, D. D., Orhan, N., Ozcelik, B.andErgun, F. (2009), "Biological activities of Vitis vinifera L. leaves". Turkish Journal of Biology, 33**,** pp. 341-348.

Rhodes, P. L., Mitchell, J. W., Wilson, M. W.andMelton, L. D. (2006), "Antilisterial activity of grape juice and grape extracts derived from Vitis vinifera variety Ribier". International Journal of Food Microbiology, 107**,** pp. 281-286.

Vaquero, M. J. R., Alberto, M. R.andDe Nadra, M. C. M. (2007), "Antibacterial effect of phenolic compounds from different wines". Food Control, 18**,** pp. 93-101.