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IN VITRO SCREENING FOR ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACT OF *VITIS VINIFERA*

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ABSTRACT

The antimicrobial activity properties of Grapes (*Vitis vinifera*) extracts were studied. Grapes extracts were obtained using solvents Methanol, N-Hexane, Water extract and Dry powder. The extracts were assayed for antimicrobial activity and bacterial growth inhibition activity by the agar well diffusion method. The results showed that all the extracts except the N - Hexane extract have minor antibacterial activity. The results also showed that Grapes extracts does not possess any such potent antimicrobial properties so that extracts cannot directly used as an antimicrobial agent. Further investigation of grapes extract should be done by other method.

Key words: Grapes, Antimicrobial Activity, Agar well diffusion method.

INTRODUCTION

Vitis vinifera (Common name: Grape) is a species of *Vitis*, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran. It is a liana growing to 35 meter tall, with flaky bark. The leaves are alternate, palmately lobed, 5–20 cm long and broad. The fruit is a berry, known as a grape; in the wild species it is 6 mm diameter and ripens dark purple to blackish with a pale wax bloom; in cultivated plants it is usually much larger, up to 3 cm long, and can be green, red, or purple. The species typically occurs in humid forests and streamside.

In Asia, the fruit is reported to contain 50.0 calories, 26.0 g H_2O, 0.5 g protein, 0.3 g fat, 12.8 g total carbohydrate, 0.9 g fiber, 0.4 g ash, 9.0 mg Ca, 20.0 mg P, 0.6 mg Fe, 6.0 mg Na, 111.0 mg K, 50.0 µg β - carotene equivalent, 0.1 mg thiamine, 0.06 mg riboflavin, 0.2 mg niacin, and 4.0 mg ascorbic acid. In further it also contains phenolic compounds as major active chemical constituents.

Anthocyanins are important polyphenols in the red grape skin, while flavan -3 - ols are the major polyphenols in the seeds. Resveratrol (3,4,5, - 3-Hydroxystilbene; trans-resveratrol) is a kind of active polyphenols in grapes (Beveridge TH *et al.*, 2005). Resveratrol (3,4,5 - Hydroxystilbene) is synthesized in grapes in response to microbial infection or stress; however, in other parts like stem may also be a source of resveratrol rather than its fruit. The resveratrol content in grapes differs according to the variety of grape.

Commercial juice products from grapes contains photochemical such as resveratrol (a polyphenol antioxidant) have been applied in medical research studies, showing potential benefits against the onset stage of cancer, platelet aggregation and other risk factors of atherosclerosis, loss of physical performance and mental acuity during aging, hypertension in humans, degenerative nerve disease, viral infections and mechanisms of Alzheimer's disease. Grape seed oil from crushed seeds is used in cosmeceuticals and skincare products for many perceived health benefits. Grape seed oil is notable for its high contents of tocopherols (vitamin E), phytosterols, and polyunsaturated fatty acids such as linoleic acid, oleic acid and alpha-linolenic acid.

The objective of the present work is to perform the *in vitro* screening for Antibacterial activity of various extract of *Vitis vinifera* (Walker AR *et al.*, 2007).

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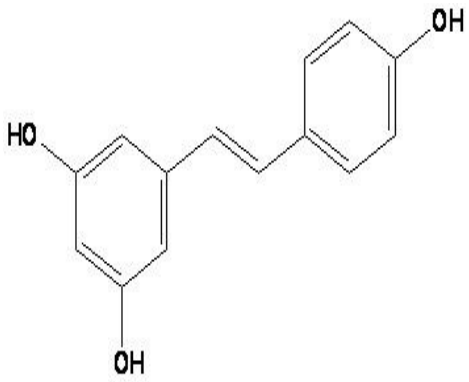
Figure 1: Liana of Vitis vinifera along with fruit (Source: Internet)

Figure 2: Structure of Resveratrol



MATERIAL AND METHOD

Preparation of Extracts

Extraction of the Vitis vinifera was carried out by the percolation method using the Soxhlet apparatus. Grapes were obtained from the market. The fruits were dried into the shade for the preparation of the dry powder.

The soxhlet methanolic extracts was obtained by shoxhlet extraction of 25g of fresh grapes in 100ml of methanol at 65 C using soxhlet apparatus. The extract was then concentrated to 20ml on a water bath and dried at room temperature.

The water extracts and N – Hexane extract were obtained by repeating the above procedure at 100^{o}C and 69^{o}C respectively. The various extracts were used for the analysis of antibacterial activities and bacterial inhibition assay.

Antibacterial activity

The antibacterial activity was determined by the diffusion method of Kirby Bauer described by Duguid *et al*., (1989). This method determines the antibacterial activity of the extracts.

Preparation of the nutrient medium

Nutrient agar medium was prepared by dissolving 2.8g of nutrient agar in 100ml distilled water. The solution was sterilized in an autoclave at 121^{o}C (1.1N pressure) for 15 min. The suspension was cooled and poured into sterile Petri dishes to solidify. The agar depth of the medium was 4.0mm (Olalla Feijoo *et al.*, 2008).

Preparation of culture and Inoculation

Pure culture of *Klebsiella pneumonia* (MTCC no. 432), *Micrococcus luteus* (MTCC no. 106), *Staphylococcus aureus* (MTCC no. 096), *Proteus vulgaris* (MTCC no. 426), *Bacillus subtilis* (MTCC no. 441), *Bacillus cereus* (MTCC no. 430), *Bacillus pumilus* (MTCC no. 1607), *Escherichia coli* (MTCC no. 443) and *Salmonella typhi* (MTCC no. 734) were obtain from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India (Walker AR *et al*., 2007), were separately used to inoculate the Petri dishes by streaking the surface of the plates in a zigzag manner until the entire surface was then covered. The inoculated plates were then incubated into the incubator at temperature specified by the MTCC for each strain.

Method for Antibacterial Assay

The extracts were dissolved in the appropriate solvent to obtain the concentration of 100µg/ml and 1000µg/ml. 4 wells were punched into the inoculated Petri plate using the 6 mm cork borer. 100ul of prepared extract were filled into the well using the micropipette using the sterile tip. The plates were incubated at the appropriate temperature. Streptomycin used as a standard drug taken as positive control (Malu SP *et al*., 2009; Satyajit D *et al.*, 2007).

The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The zones were measured.

RESULTS & DISCUSSION

Here all the test samples were analyzed by the Agar well diffusion method for antimicrobial activity. From the Table 1 We found that none of test sample shown activity at lower concentration i.e. at 100 µg/ml concentration. So we can conclude that none of test sample is as potent to inhibit the microbes at lower concentration i.e. 100µg/ml. Further at higher concentration i.e. at 1000 µg/ml Methanolic extract gave activity in the *Bacillus* species but still it was not potent that can be use as antimicrobial agent. Dry powder and water extract also gave activity against Bacillus species but comparatively less than the methanolic extract. N – Hexane had shown activity neither at lower concentration nor at higher concentration.

Table 1. Result for Antimicrobial assay of various extract of *Vitis vinifera* using Agar well diffusion method

Microbial Strain	Conc.	Methanolic Extract	Dry Powder	Water Extract	N – Hexane Extract	Streptomycin
K. pneumonia	A	–	–	–	–	++
	B	–	–	–	–	+++
M. luteus	A	–	–	–	–	++
	B	+	–	–	–	+++
S. aureus	A	–	–	–	–	++
	B	–	–	–	–	+++
P. vulgaris	A	–	–	–	–	++
	B	–	–	–	–	+++
B. subtilis	A	–	–	–	–	++
	B	+	+	+	–	+++
B. cereus	A	–	–	–	–	++
	B	+	–	+	–	+++
B. pumilus	A	–	–	–	–	++
	B	+	+	+	–	+++
E. coli	A	–	–	–	–	++
	B	+	+	+	–	+++
S. typhi	A	–	–	–	–	++
	B	–	–	–	–	+++

Concentration of Extract: A = 100µg/ml, B= 1000µg/ml. **Concentration of Streptomycin**: A = 100µM, B= 1000µM

Sign	Zone of Inhibition (in mm)
+	1 – 9
++	10 – 14
+++	15 or above
–	No Inhibition

CONCLUSION

From the result we can conclude that none of the sample is as potent to inhibit the microbial strain used in the assay. So the further investigation should be done using more purified extract by another sensitive method like Microtitre plate based Antimicrobial assay technique.

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