RESEARCH ARTICLE

Pharmacological Effect of Grape Juice on Bacteria Isolated From Sputum of Ambrose Alli University Students

Obiazi Helen. A*

ABSTRACT: Grape (Vitis vinifera L. Vitaceae) is cultivated world-wide, which has both phytochemical and pharmacological effects through its most active constituents known as phenolic compounds. The aim of the study was to assess the pharmacological effect of grape juice on bacteria isolated from sputum of Ambrose Alli University students. Sputum samples were investigated using nutrient agar, MacConkey agar and Mannitol salt agar for primary culture and incubation was carried out at 37°C for 24hrs. Further identification was done using standard bacteriological procedures and pharmacological effects was ascertain by antimicrobial screening. The result shows Klebsilla spp was sensitive at 1000mg/ml (10mm), 500mg/ml (12mm) and 250mg/ml (8mm). Staphylococcus spp was sensitive at 1000mg/ml (12mm), 500mg/ml (8mm) and 250mg/ml (8mm) while for Streptococcus spp was sensitive at 1000 (6mm). However, Minimum Inhibitory Concentration (MIC) of grape extracts on isolates ranged from 1000-500mg/ml for Klebsilla spp and Staphylococcus spp while 500-250mg/ml for Streptococcus spp. In conclusion. this research shows the inhibitory effect of grape juice extracts on the growth of Staphylococcus aureus, Streptococcus spp and Klebsiella spp. However, this study has revealed that grape juice has several active agents that are inhibitory to microorganisms.

Key Words: *Grape Juice , Bacteria, Sputum.*

INTRODUCTION

Phytomedicine, which has historically been an important aspect of traditional medicine in non-industrialized countries, is now becoming an integral part of healthcare in industrialized countries. Plants are the source of thousands of new phytochemicals, and different strategies can be applied to improve the yields of bioactive metabolites in the plant and to obtain chemically standardized extracts (Mora-Pale et al., 2014; Demarque et al., 2015). Along with conventional methods, numerous new methods have been established but till now no single method is regarded as standard for extracting bioactive compounds from plants. The efficiencies of conventional and non- conventional extraction methods mostly depend on the critical input parameters; understanding the nature of plant matrix and chemistry of bioactive compounds (Azmir et al., 2013).

Grape (Vitis vinifera L. Vitaceae) is cultivated world- wide (Afzalzadeh et al., 2015), and it is the world's largest fruit crop with an annual production of more than 67 million tons (Fontana et al., 2013). In recent years, several important reviews have described the phytochemical and pharmacological effects of grape and the active constituents in different parts of the fruit, including skin, seeds, pomace and stems (Tang and Chan, 2014). The most important active constituents of grapes are phenolic compounds (Tang and Chan, 2014). In 2009, Amico et al. reported that the main constituents of the grape stem ethanolic extract are: two triterpenoid acids, oleanolic and betulinic acids; a stilbenoid, daucosterol; E-resveratrol and its dimer E-⇓-viniferin; gallic acid as a simple phenol; catechin and gallocatechin (flavanols); four 6⇓-O-acyldaucosterols; and five 1,2-di-O-acyl-3-O-⇓-D-galactopyranosyl glycerols (Amico et al., 2009). V. vinifera seeds were found to contain considerable quantities of gallic acid in addition to smaller amounts of p-coumaric, caffeic and ferulic acids. They contained greater amounts of both gallic and p-coumaric acids than the seeds of other fruit varieties (Weidner et al., 2013). When Corinthian currants (V. vinifera L., var. Apyrena) were examined, five anthocyanidin-3-O-glucosides were identified and quantified, with malvidin-3-O-glucoside, peonidin-3-O-glucoside and cyanidin-3-O-glucoside be- ing the most abundant glucosides (Chiou et al., 2014).

The most abundant flavanols in red grape pomace extract (V. vinifera L. cv. Malbec) were (+)-catechin and (-)-epicatechin with malvidin-3-glucoside being the most abundant anthocyanin. Furthermore, for the first time, piceatannol, a stilbene analogue to resveratrol, was identified and quantified in grape pomace (Antoniolli et al., 2015). It was reported that the phenolic composition of V. vinifera L. fruits is highly dependent on the grape variety (de la Cerda-Carrasco et al., 2015).

Literature review has shown that grape has many phytochemicals and this study aimed to assess the pharmacological effect of grape juice on bacteria isolated from sputum of Ambrose Alli University students.

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MATERIALS AND REAGENTS

The materials and reagents used during the course of this research include: weighing balance, beakers, conical flasks, autoclave, petri-dishes, 70% ethanol, non-absorbent cotton wool, aluminium foil, test tubes, wire loops, incubators, microscope, nutrient agar, cover slip, Mannitol salt agar, MacConkey agar, peptone water and distilled water.

STERILIZATION

Autoclavable materials such as agar and broth were aseptically sterilized in an autoclave at 121 °C for 15 minutes. Petri dishes, beakers, McCartney bottles, pipette, test tubes, filter papers and other metal apparatus such as spatula and forceps were sterilized using hot air oven at a temperature of 160 °C for 1 hour. The wire loops were sterilized by heating in the blue flame of the bunsen burner until red-hot and allowed to cool before using 70% alcohol to

Department of Microbiology, Faculty of Life Science, Ambrose Alli University, Ekpoma

*Correspondence: Obiazi Helen. A, Department of Microbiology, Faculty of Life Science, Ambrose Alli University, Ekpoma, Tel:+2348056400152, E-mail: Obiazihelen@gmail.com**Received: October 12, 2020, Accepted: October 28, 2020, Published: November 04, 2020*

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swab the work bench area to prevent contamination. The process was carried out aseptically.

PREPARATION OF CULTURE MEDIA

All culture media were prepared according to manufacturers' instructions and autoclaved at 121 °C for 15 mins.

COLLECTIONS OF SAMPLES

Fresh Grape juice were obtained from surroundings of homes and gardens in Ekpoma, Edo State, Nigeria The variety was chosen because it is widely used in all parts of the country as spice, condiments and in soup making.

PREPARATION OF ORGANISMS

Serial dilution was carried out on the isolates collected from isolated food sources and 10-4 of the serial dilution was used for the sensitivity testing.

RECONSTITUTION OF EXTRACT

The dried extracts were reconstituted by dissolving 5g each of the extract in 50ml ethanol and 50ml distilled water. The solution was filtered using the sterile whatman no1 filter paper. The stock solution was sterilized by filtration through filter paper to remove impurities and other contaminants. The stock solution was further dissolved at different concentration and it was then stored in sterile universal bottles and refrigerated for further analysis.

IDENTIFICATION OF TEST ORGANISMS

All isolates for this study were identified by their colonial appearance on the media, gram staining reaction and biochemical tests.

GRAM STAINING

The growth on the culture plate was carefully placed on a sterile, grease-free microscope slide and allowed to air-dry. It was fixed by passing it over the pilot flame of the Bunsen burner three times.

The fixed smear was flooded with Crystal violet for 1 minute before washing off with tap water. Lugol's iodine was added and washed up after about 1 minute and subsequently decolourised rapidly using acetone and washed off immediately. Neutral red (Counter stain) was then added and washed off after about 2 minutes. The slides were then placed in a draining rack for the smear to air dry. After drying, a drop of immersion oil was applied on the smear and viewed microscopically using oil immersion objectives (Cheesbrough, 2006)

BIOCHEMICAL CHARACTERISATION AND IDENTIFICATION

CATALASE TEST

This test helps to differentiate staphylococci from streptococci. Few colonies of the organism were emulsified in normal saline on a clean grease free slide placed in a petri dish. Two drops of H2O2 were added to the suspension and the petri dish covered. Gas bubbles were observed for some and were not observed in others (Ochei and Kolhatkar, 2000).

CITRATE TEST

This test is one of the important tests used in the identification of enterobacteria. A light suspension of the organism was made in normal saline and inoculated into Koser's citrate medium with a straight wire. Growth and development of blue colour indicated by turbidity in Koser's medium in Simmons agar indicates positive result meaning that citrate has been utilized (Ochei and Kolhatkar, 2000).

COAGULASE TEST

This test is used to differentiate Staphylococcus aureus from other staphylococci species. Human plasma was diluted 1 in 10 in saline. Into each of the three test tubes for each sample, 0.5ml of the diluted sample was added. Five drops of 18-24 hour broth culture of the test organisms was added to the 1st test tube. Five drops of 18-24 hour broth culture of Staph. aureus was added to test tube 2 and five drops of sterile broth was added to test tube 3. They were all mixed gently and incubated in a water bath and examined for clot after one hour, two hours and at 30 minutes interval for up to 6 hours. The tubes were observed for clot. The positive control produced clot within 1 hour and the negative control did not produce any fibrin clot (Ochei and Kolhatkar, 2000).

OXIDASE TEST

This test is employed to aid in the identification of Pseudomonas, Neisseria, Vibrio and other groups. A few drops of oxidase reagent was added to a few colonies on the culture plate. Colour change of blue to deep purple was looked out for within 5-10 seconds (Ochei and Kolhatkar, 2000).

INDOLE TEST

This test for Indole production is used as an aid in differentiation of Gram negative bacilli. The organism was grown in peptone water overnight. A few drops of Kovac's reagent were added to the overnight peptone water culture. Colour change was looked out for. Red colouration indicates positive indole production (Ochei and Kolhatkar, 2000).

ANTIMICROBIAL SCREENING FOR RECONSTITUTED EXTRACT

Two methods were employed for the antimicrobial testing which are the, Agar diffusion method and Disc diffusion method.

DISC DIFFUSSION METHOD

The locally prepared sterile discs were soaked in the water extract for some hours and nutrient agar medium was poured in sterile Petri Dishes and it was allowed to solidified.1ml of the test organisms was placed on the solidified agar and it was spread all over the surface of the agar. The soaked disc was picked using sterile forceps and it was dropped on the surface of the agar. The plates were incubated at 370 C for 24 hours. Sensitivity of the organisms was recorded.

RESULTS

Table 1 shows the anti-bacterial sensitivity pattern of grape extracts on isolates from sputum, in which Klebsilla spp was sensitive at 1000mg/ml (10mm), 500mg/ml (12mm) and 250mg/ml (8mm). Staphylococcus spp was sensitive at 1000mg/ml (12mm), 500mg/ml (8mm) and 250mg/ml (8mm) while for Streptococcus spp was sensitive at 1000 (6mm). Table 2 shows the minimum inhibitory concentration (MIC) of grape extracts of isolates which ranged from 1000-500mg/ml for Klebsilla spp and Staphylococcus spp while 500-250mg/ml for Streptococcus spp.

TABLE 1: Anti-bacterial Sensitivity pattern of Grape extracts on isolates from sputum

Isolates	1000mg/ml	500mg/ml	250mg/ml	+ve Control	-ve Control
Klebsilla spp	S(10mm)	S(12mm)	S(8mm)	+	-
Staphylococcus spp	S(12mm)	S(8mm)	S(8mm)	+	-
Streptococcus spp	S(06mm)	R(-)	R(-)	+	-

TABLE 2: Anti-bacterial Sensitivity pattern of Grape extracts on isolates from sputum

Isolates	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml
Klebsilla spp	-	+	++	+++	+++++
Staphylococcus spp	-	+	++	++	+++
Streptococcus spp	-	-	++	+++	++++

TABLE 3: Biochemical Test of isolated Microorganism

Isolates	Cultural characteristics					Biochemical analysis				Sugar Fermentation				Organism
	Gram	Shape	Shape of colony	Consistency	Colour	Catalase	Coagulase	Oxidase	Indole	Glucose	Sucrose	Lactose	Maltose	
1	+	Cocci	Convex	Moist	Colourless	+	+	_	_	+	NA	NA	NA	*Staphylococcus aureus*
2	-	Rod	Irregular	Moist	White	-	-	+	-	A	-	-	-	*Streptococcus* spp
3	+	Cocci in cluster	Circular	Moist	Colourless	-	-	-	-	A/G	A/G	A/G	A/G	*Klebsiella* spp

DISCUSSION

The present study was designed to determine the antimicrobial activity of extract of grape juice. The isolates were confirmed as Staphylococcus aureus, Streptococcus spp and Klebsiella spp by cultural and staining characteristics and biochemical test. The result of the antimicrobial activity of a grape extract was determined by diameters of inhibition zones are present in table 1. It indicated that the diameters of inhibition zones varied from 2mm to 8mm for positive control and 6mm-12mm for grape extracts.

Studies have reported many natural plant extracts with anti-bacteria activity, including garlic, broccoli, cranberries, and green tea (Fahey et al., 2002). Grapes (Vitis vinifera), well known for their high levels of antioxidants and polyphenols, have also shown promise as novel antimicrobial agents. A few studies have already reported the anti-bacteria activities of grape seed and juice, including an active chemical constituent (e.g., resveratrol, a stilbene from red wine) (Nohynek et al., 2006). However, no effort has been made to evaluate the grape skin or different grape types (e.g., table and muscadine grapes). For example, muscadines (Vitis rotundifolia) contain significantly higher levels of phenolics than commercial table grapes in addition to possessing some unique forms of these compounds (Talcott and Lee, 2002).

However, little is currently known about the antibacterial properties these fruits possess, making them prime candidates for study. In addition, it is believed that the high complexity of bioactive compounds present in these products and their broad range of activity over a number of microorganisms may make it difficult for microbes to acquire resistance during treatment (Vattem, et al., 2005).

The results of the antimicrobial activity of grape extract was determined by MICs are represented in table 2. The MIC tests of grape juice against three microbial species was carried out using the broth dilution techniques. Minimum inhibitory concentration (MIC) of grape extracts of isolates which ranged from 1000-500mg/ml for Klebsilla spp and Staphylococcus spp while 500-250mg/ml for Streptococcus spp.

CONCLUSION

This research shows the inhibitory effect of grape juice extracts on the growth of Staphylococcus aureus, Streptococcus spp and Klebsiella spp. However, this study has revealed that grape juice has several active agents that are inhibitory to microorganisms. It is therefore recommended that the public should be enlightened on this beneficial effect, thereby promoting the consumption of grape juice.

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Nil

CONFLICTS OF INTEREST

There is no conflict of interest.

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