THE ANTIBACTERIAL EFFICACY OF CRUDE *Vitis vinifera* (GRAPE) SEED EXTRACT AGAINST *Staphylococcus aureus* AND *Escherichia coli*

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ABSTRACT

Antimicrobial is coined from the Greek words 'anti' meaning against, 'mikros' referring to small, and 'bios' meaning life. Antimicrobials are agents that act against all types of microorganisms. Often, plant extract is one of the main ingredients in making these antimicrobials. In this study, the researchers used ethanolic crude extract of the Vitis vinifera (grape) seed to determine its efficacy as an antibacterial agent. The extracts were tested against Staphylococcus aureus and Escherichia coli using cylinder plate assay. Zones of inhibition produced by the fruit extracts were measured, then statistical treatment to determine its significance was employed. There was a considerable difference between the represented value of the positive and the known concentration. The positive control, having a mean value of 50ppm, is significantly the highest concentration for the given extract. However, after testing the antibacterial property of the grape seed extract, the result showed that on the given concentration, there was no zone of inhibition, which means it is not effective against Staphylococcus aureus and Escherichia coli. Therefore, Vitis vinifera (grape) seed ethanolic crude extract cannot be used as an antibacterial agent against Staphylococcus aureus and Escherichia coli.

***Key words**: Antimicrobial, Ethanolic crude extract, Vitis Vinifera, Staphylococcus aureus, Escherichia Coli, cylinder plate assay, Zone of inhibition*

INTRODUCTION

Vitamins are substances that the body needs, and eating fruits is one of the best ways to get all the vitamins a person needs each day. One of the fruits that has become a favorite of many is grapes. Grape is used as a medicine generally because it could cure some diseases or can be a food supplement. In this paper, the researchers are looking for more contribution it could give.

The common grape (*Vitis vinifera)* which is in the family *Vitis* is a grape vine that originated on the region of Mediterranean, Middle part of Europe and Southwest of Asia, from Morocco, North of Portuga, and to the south of Germany and parts of Eastern and Northern Iran. The common grape is cultivated on regions that produces wine around the world except on Antartica (Bot, 2010).

Common grape is liana that grows 32 centimeter in length around 35 yards, consist of a bark that is flaky, and it is considered as an important and a principal fruit products in the world (Akaberi, 2016). A dietary supplement from grape, the grape seed extract is well known. According to studies, it contains the necessary minerals, vitamins, as well as polyphenols. It contains an abundance of compounds that is phenolic in nature, which has properties that can be anti – inflammatory, anti – bacterial, cancer preventing, anti – viral, neurologic, hepatic, cardio protecting effects, as well as an age slowing and anti-oxidant. Oil extracted from grape seeds is used in cosmetic, culinary, pharmaceutical, and medical purposes (Kekuda et al., 2014).

Todar (2012) stated that *Staphylococcus aureus* are aerobic gram-positive bacteria that is microscopically grape-like in clusters and grows at 15 to 45 degree of temperature which has a perfectly spherical cell about 1 micrometer in diameter. Its common isolates are usually found on the normal flora of the human skin, nose, and considered as a one of the major causes of infections after a surgery. *Escherichia coli* (E. coli) a Gram-negative organism shaped as rod is facultative in nature and anaerobic. Mostly *Escherichia coli* strains reside in the

human and animals intestinal flora as a common resident of the gastrointestinal tract (Biotechnol, 2010).

In this research the researchers used an alternative medicine rather than an antibiotic, and that is the grape seed extract which has an antibacterial activity due to its phenolic compounds. It has also been recognized for its beneficial role in human health which exhibits bioactivities that leads the researchers to adapt the study of Xia et al., (2010), regarding the antimicrobial activity of grape seed extract, and to endorse new methodologies to the future researchers.

METHODOLOGY

Research Design

The researchers focused on the efficacy of *Vitis vinifera* (grape) extract of seed as anti-bacterial agent against *Escherichia coli* and *Staphylococcus aureus*. It is an experimental study and the two bacteria are the two quantitative variables of the researchers. The methods and procedures of this study were adapted from the previous study of Kekuda (2014), the "Antimicrobial Activity of Grape Seed Extract Which Employed the Agar Well Diffusion Method". Methanolic crude extract of grape seed was evaluated which helped the researchers in determining its antimicrobial activity on two microorganisms, *Staphylococcus aureus* and *Escherichia coli.*

Research Locale

The researchers bought 30 bunches (20 kilograms) of fresh grapes at a supermarket in Calamba, City and performed the experimental test at one of the laboratories in Lyceum of the Philippines University Laguna, St. Cabrini School of Health Sciences.

Materials

The materials that the researchers used are as follows: petri dish, inoculating loop, alcohol lamp, cork borer, beaker, graduated cylinder, Erlenmeyer flask, sonicator, microliter syringe, vernier caliper, and the two microorganisms-- *Staphylococcus aureus* and *Escherichia coli*. Laboratory gown was worn to protect the clothes of researchers as well as mask and gloves to protect them from the dangerous chemicals in the laboratory.

Chemicals and Reagents

The reagents that the researchers used were *Vitis vinifera* (grape) seed extract, which is the test subject, Cefuroxime (positive control), Dimethyl sulfoxide (negative reagent), and distilled water.

Preparation of Grape Seed Extract

Fully ripe grapes were purchased from a local shop in market of Calamba city. The grapes were macerated, and seeds were separated. The seeds were washed thoroughly using a purified water and then air dried. The seeds were powdered in a blender. 90 grams of grape seed became 65 grams when it was powdered. It was transferred to a conical flask containing 150 ml of 80% ethanol and then stirred well. The flask was left aside for 120 hours or for 5 days and was stirred occasionally. The content of flask was filtered through Whatman No. 1 and evaporated to dryness of a room temperature.

Antibacterial activity of Grape Seed Extract

Agar well diffusion method was performed to investigate antibacterial efficacy of grape seed extract against two bacteria-- *Escherichia coli* and *Staphylococcus aureus*. 24 hours old Mueller Hinton Agar cultures of test bacteria were swabbed uniformly on sterile Mueller Hinton Agar plates. The procedure took place when agar had been prepared. The bacteria was inoculated and streaked to agar

plates. Then, the researchers opened the middle of the plate using cork borer. An antibiotic was applied (cefuroxime as positive control and grape seed extract (GSE) and the Dimethyl sulfoxide (DMSO) as negative control). It was incubated for 24 hours to measure the zone of inhibition using caliper.

RESULTS AND DISCUSSION

This chapter presents the gathered and analyzed data of the research by measuring the zone of inhibition of Grape Seed Extract, Cefuroxime (positive control) and Dimethyl Sulfoxide (negative control) against the two microorganisms.

Table 1. Measurement of Zone of Inhibition for the assessment of the antibacterial efficacy of Grape Seed Extract, Dimethyl Sulfoxide and Cefuroxime against *Staphylococcus aureus*

Trials	Cefuroxime (positive control)	Interpretation	Dimethyl Sulfoxide (negative control)	Interpretation	Grape Seed Extract	Interpretation
Trial 1	2.82	Susceptible	0	Not effective	2.49	Susceptible
Trial 2	3.34	Susceptible	0	Not effective	1.91	Susceptible
Trial 3	2.78	Susceptible	0	Not effective	1.82	Susceptible

Range: Resistant = ≤1.4; Intermediate = 1.5 – 1.7; Susceptible = ≥1.8

Table 1 shows the antibacterial efficacy of grape seed extract, Cefuroxime (positive control) and Dimethyl Sulfoxide (negative control) against the microorganism *Staphylococcus aureus.* The zone of inhibition of Cefuroxime in *Staphylococcus aureus* in trial two was bigger which is 3.34 cm out of the three trials while the zone of inhibition of grape seed extract in the same microorganism was bigger in trial one which is 2.49 cm.

All bacterial strains were said to be susceptible to cefuroxime and grape seed extract respectively because they fall within the range

of susceptible which is ≥*1.8.* The antibacterial property of grape seed extract is said to be due to the presence of polyphenols which was agreed by the study done by Howell, Su, & D'Souza (2012), which determined that among the tested agents, Grape Seed Extract (GSE) at 1 and 5 mg/ml concentration was established resulting in colony reduction of both strains after 2 hours at 37°C. Furthermore, a study conducted by Al-Habib et al. (2012), reported that the anti – bacterial effect the seed extract of grape acts on MRSA by disruption of its cell wall observed using scanning and transmission electron microscopy. In which that activity is found out to be by the action of potent polyphenols found on the grape seed extract.

Table 2. Measurement of Zone of Inhibition for the assessment of the antibacterial efficacy of Grape Seed Extract, Dimethyl Sulfoxide and Cefuroxime against *Escherichia coli*

Trials	Cefuroxime (positive control)	Interpretation	Dimethyl Sulfoxide (negative control)	Interpretation	Grape Seed Extract	Interpretation
Trial 1	2.93	Susceptible	0	Not effective	2.23	Susceptible
Trial 2	2.83	Susceptible	0	Not effective	1.86	Intermediate
Trial 3	3.01	Susceptible	0	Not effective	1.77	Intermediate

Range: Resistant = ≤1.6; Intermediate = 1.7 – 1.9; Susceptible = ≥2

Table 2 shows the antibacterial efficacy of grape seed extract, Cefuroxime (positive control) and Dimethyl Sulfoxide (negative control) against the microorganism *Escherichia coli.* The zone of inhibition of Cefuroxime in *Escherichia coli* in trial three was bigger which is 3.01 cm out of the three trials, while the zone of inhibition of grape seed

extract in the same microorganism was bigger in trial one which is 2.23 cm.

All bacterial strains for Cefuroxime was susceptible because it falls within the range ≥2, but bacterial strains for grape seed extract showed intermediate for trials two and three. This may be because other *Escherichia coli* strains needed longer time for the medication to take effect, and the concentration of the crude extract is not sufficient to the extent of killing other Extended Spectrum Beta Lactamase (ESBL) *Escherichia coli* since we only used one concentration. Nevertheless, grape seed extract still inhibited the said bacteria. According to Shrestha et al. (2012), using structure activity correlation assay, it showed that the hydroxyl found on the compound of grape seed extract was found to be effective against *Escherichia coli* while the benzene ring was effective against *Staphylococcus aureus*. In a study conducted by Kandasamy et al. (2016), grape seed extract was proven to be a bacterial reducing agent and can exhibit moderate zones of inhibition ranging from 11 to 14 millimeter against the common clinical isolates and 2 to 4 millimeter against the drug resistant strains from the concentrations of the extract ranging from 2mg/ml to 20mg/ml.

CONCLUSION

After conducting the experiment and gathering all necessary data, the researchers observed that *Staphylococcus aureus and Escherichia coli* became affected with the grape seed ethanolic crude extract at a given concentration. They were affected because the agar plate had been made in advance and had been put in the refrigerator. The experiment was done one week late using the agar. There was also a manifestation of zone of inhibition which shows a sudden change in the appearance of the grape seed extract itself. There were bacteria present, but they have not grown enough to be visible. Based on the results obtained, the researchers conclude that the *Vitis vinifera* (ethanolic crude extract) is an effective antibacterial agent.

RECOMMENDATIONS

After conducting all trials and experiments, the researchers noted points for the future researchers to modify and improve. First, the researchers endorse to utilize other parts of *Vitis vinifera* (grape) such as peel and fruit extract for further testing against other bacteria of medical importance.

The researchers recommend to try different methods that will lead into the conclusion that *Vitis vinifera* (grape) seed fruit ethanolic crude extract is possibly effective in inhibiting microorganisms. Specifically, the researchers propose to the future researchers that ethanolic crude extract of grape seed is more extent on aerobic gram positive bacteria groups like *Staphylococcus aureus* whereas aerobic gram negative bacteria like *Escherichia coli* was inhibited to the least extent. Using Cefuroxime as both of its positive control will be efficient when it comes to antimicrobial testing because it is highly active against gram-positive and gram-negative cocci. The researchers also propose to use other fruit related to *Vitis vinifera* (grape) that may contain antibacterial property.

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