



Evaluation of the Antibacterial Activity of Various Concentrations of Raisin Extract against Streptococcus Mutants: An In-Vitro Study

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Abstract

Background: Dental caries is one of the most common and costly diseases in the world. Although dental caries is rarely life threatening, it is a major problem for health service providers. *S. mutans* is considered as the pioneer organism in dental caries. Fruits like grapes and pomegranate have been found effective against *S. mutans*. **Objectives:** To find out minimum inhibitory concentration of black raisins extract against *S. mutans* and to assess whether the black raisins extract has antibacterial efficacy on *S. mutans*. **Methods:** In this in vitro study, compound isolatin of raisin extract was done using chloroform, hexane and methanol, prepared in Department of Pharmacology, Dayananda Sagar Institutions. Minimum inhibitory concentration of raisin extract was determined using optical density method. Evaluation of antibacterial activity was done using well diffusion method. **Results:** Minimum inhibitory concentration of raisin extract was observed at a concentration of 125 µg/well at which an inhibition rate of 48.65% was observed. Minimum zone of inhibition of 16±0.0mm was observed at a concentration of 2.5 µg of raisins extract. **Interpretation and conclusion:** Raisins contain polyphenols, antioxidants, flavonoids and iron that may benefit overall human health. It has an MIC value of 125 µg/ml at which the rate of inhibition is 48.65%. The structural diversities and pronounced biological activities of compounds in raisins indicate that raisins are worthy of further studies that may lead to the identification of new functional constituents.

KEYWORDS

Raisin Extract, Streptococcus Mutants, Antibacterial Efficacy, Dental Caries

1 | INTRODUCTION

Dental caries is one of the most common non communicable and costly diseases in the world. Although dental caries is rarely life threatening, it is a major problem for health service providers.¹ According to Global Oral Health Data Bank, prevalence of dental caries varies from 49% to 83% across different countries.² An enhanced understanding of the role

of the microorganisms in dental caries is needed to reduce the prevalence of caries. *S. mutans* is considered as the pioneer organism in dental caries. *S. mutans* play a key role in the etiology of dental caries because it can adhere to the enamel salivary pellicle and to other plaque bacteria.³ Addition of antiplaque or antibacterial agents to dental health care products has been of value in controlling dental caries.

Various products like chlorhexidine, sodium fluoride, xylitol and triclosan have been found very effective in controlling *S. mutans* count.⁴ Extracts containing polyphenols of plant origin gained more attention

of researchers for their use against drug-resistant pathogens.⁵ Antibacterials or antibiotics from these sources have been found to be

more efficient with fewer side effects and less cost of production.⁶ Plant-based phenolic metabolites are beneficial to human health because of their potent antioxidation and wide range of pharmacologic properties such as antioxidant, anticancer, and platelet aggregation inhibition activities. It is important to stress more on natural products than artificial drugs. Going green has major benefits to patients and also environment.

Fruits like grape contain various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Polyphenols are the most important phytochemicals in grape because they possess many biological activities and health promoting benefits. Polyphenolics from grapes and red wines attracted the attention of scientists to define their chemical composition and their properties for human health.

Black grape (*Vitis vinifera*) skin is a great source of phenolic compounds, which contains simple compounds (monomers) to complex tannin type substances (oligomers and polymers). The various classes of negatively charged polyphenols that have been identified in grapes, such as phenolic acids (benzoic, hydroxycinnamic acids), stil-bene derivatives (resveratrol), flavanols (catechin, epicatechin), flavonols (kaempferol, myricetin), anthocyanin and many more.

These polyphenols boast many beneficial effects on human health such as inhibition of free radical damage, antibacterial, antifungal, increase cardiovascular health, anticarcinogenic and anti-inflammatory actions on human health.⁷ Grape as a whole is generally underutilized and thrown away by the wine factory as waste products. Grape skins are rich sources of anthocyanins, hydroxycinnamic acids, flavanols and flavonol glycosides in which flavanols are mainly present in the seeds.⁸

Anthocyanins give black grapes their color. It is present in a huge amount as compared to other polyphenolic compounds. The higher amounts of dimers and trimers of epicatechin which possess a higher antibacterial activity than monomer ones.⁹ Thus black grape is found to have both antifungal and antibacterial activities.¹⁰

Catechins, along with other polyphenols are highly negatively charged phytochemicals, that give the antifungal property to black grape. The phenolic compounds from different parts of grape have shown different antibacterial effects. antibacterial activity of fermented grape was either as effective as or significantly better than whole fruit grape extracts.¹¹ Various compounds in grape are being studied for their activity against *S. mutans*.

There is scarce literature reporting the antibacterial activity of raisins extract against *S. mutans*. The purpose of this study was to assess antibacterial activity of chloroform soluble black grape raisins extract against *S. mutans*.

2 | METHODOLOGY

Study Design: In vitro study

Study Setting: The raisin extract was prepared in Department of pharmacology, Dayananda Sagar Institutions. The calculation minimum

inhibitory concentration of raisin extract was done at In vivo Bioscience lab, Magadi road, Bangalore.

Materials used:

Black Grape (*Vitis V iniferous*E thanol 99% v/v)

Hexane (80% v/v)

S. mutans (25175) Ciprofloxacin (0.1 mg/mL)

Distilled Water

Soya bean Casein Digested agar plates

Compound isolation:

The dried and ground raisins (500 mg were extracted with one litre of ethanol three times by maceration, for up to three days. The resultant extracts were combined and concentrated in vacuo at 40 degree celsius. The concentrated extract was suspended in 90% ethanol (250 ml), and then partitioned with 250 ml of hexane three times to afford hexane soluble syrup on drying. Next, the aqueous methanol extract was partitioned with 250 ml of chloroform three times to give a chloroform soluble extract as per study by Zhu et al.¹²

Estimation of minimum inhibitory concentration:¹³

It was done by mixing 90µl test compounds of different test concentration with 10µl Inoculum and was kept in 96 well plates. For Control, 90µl peptone broth without drug was used. The *S. mutans* treated plate was incubated at 37°C for 24 to 48 hours and optical density at 590 nm was measured using a tecan plate reader. Minimum inhibitory concentration of grape extract giving 50% After incubation 20µl Resazurin (1000µg/ml was added to each well and incubated for 4hr to confirm the viability of culture.

Evaluation of ANTIBACTERIAL activity by Well Diffusion Method:

The antibacterial activity was determined by well diffusion technique. It was performed on soya bean casein digested agar plates. *S. mutans* (ATCC 25175) was used as test organism and Ciprofloxacin (100 µg/mL) was used as standard.¹⁴ Distilled water was used as a control. *S. mutans* (100µl) was inoculated on soya bean casein digested agar plates (90 mm). Raisins extract (25 µl), standard ciprofloxacin (25µl) and Control (25µl) for *S. mutans* were added to the 5mm well on agar plates. The treated plates with *S. mutans* were incubated at 37°C for 24-48 hrs. The Minimum inhibitory concentration of raisin extract was determined by the ability of each substance to inhibit the growth of *S. mutans* around the 90 mm wells in soya bean casein digested agar plates. (Fig: 1)

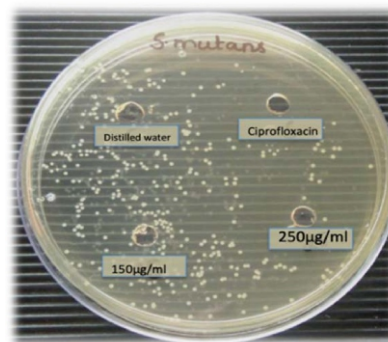


Fig: 1 Soya Bean Case in Digested Agar Plate showing Zone of Inhibition

3 | RESULTS

Various concentrations of raisins were used to find the minimum inhibitory concentration of the extract. At 15.62 µg/well concentration, 15.19% inhibition was found for raisins extract. The concentration of raisins extract was gradually increased up to a concentration of 1000 µg/well at which the inhibition of 75.98 % was observed. Minimum inhibitory concentration of raisin extract was observed at a concentration of 125 µg/well at which an inhibition rate of 48.65 % was observed.

For ciprofloxacin, 50% inhibition or an MIC value was observed at a concentration of 0.5 µg/well.

Minimum zone of inhibition of 16±0.0 mm was observed at a concentration of 2.5 µg of raisins extract. There was no zone of inhibition found at a concentration of 1.5 µg of raisins extract. Minimum zone of inhibition of 25±0.0 mm was observed at a concentration of 2.5µg for ciprofloxacin.

4 | DISCUSSION

The presence of anthocyanins, in a huge amount as compared to other polyphenolic compounds gives black grapes their colour. The amount of total polyphenols in the black grape varieties is higher as compared to that of green grape, due to the presence of the anthocyanins. The antibacterial property is also aided by the presence of polyphenols like oleanolic aldehyde, linoleic acid, linolenic acid, botulin, betulinic acid, 5-(hydroxymethyl)-2-furfural, rutin, b-sitosterol, and b-sitosterol glucoside were identified by comparing their physical and spectroscopic values. as an antibacterial agent, these polyphenols can penetrate the semi permeable cell membrane where they react with the cellular proteins. The intention of the current study was to check whether higher concentration gives better efficacy against S mutans. This study also evaluated the MIC of raisins extract collectively against S. mutans.

A study conducted by Cruz. J.F and Zhu. M¹² using the same method as the present study assessed the MIC of various compounds in raisins extract individually. The results of the study showed that various polyphenols in raisins showed different MIC values against S. mutans. The MIC values for different polyphenols were Oleanolic acid (625 µg/ml), Lineoleic acid (488 µg/ml), Rutin (250 µg/ml) and for 5 Hydroxymethyl-2- furfural (31 µg/ml). The results of the current study could not be compared with this study because current study compared the MIC value of raisins extract collectively.

A study was conducted by Yadav D, Kumar A and Mishra D¹³ to find the antibacterial activity of black grape. The results of thier study showed that the extracts showed MIC of 260 µg /ml polyphenols against S. aureus and E. aerogenes. MIC of 540 µg/ml was found against E.faecalis. In this study the MIC value against S. mutans was not determined. Thus results of the current study could not be compared with this study.

In the present study we found an MIC value of 125 µg/ml against S.mutans. The zone of inhibition was determined by well diffusion technique using soya bean casein digested agar plates and it was found to be 16 mm after incubating it for 48 hours at 37°C. The results of the current study could not be compared with that of other studies as this is the first study of its kind which compared the MIC value of raisins extract collectively.

Limitations of the study:

Firstly, it was an in vitro study; the results cannot be extrapolated to in vivo situation. Secondly, in this study the MIC value for polyphenol components was determined collectively and further studies determining the MIC of individual polyphenol compound in the black raisin extract need to be conducted.

Further studies are recommended using Innovative technologies to obtain the pure black raisins extract and in vivo studies has to be conducted to find the efficacy of black raisins extract. Assessing the antibacterial efficacy against S. mutans is a surrogate end point but the true end point is the change in dental caries that has to be detected or find out using longitudinal studies.

Table1: Inhibitory activity of test compounds against test organism

Test Organisms	Test Compounds	Concentration per well	Zone of inhibition (mm)
Streptococcus mutans	Control	-	-
	Ciprofloxacin (Standard)	2.5µg	25±0.0
	Streptococcus mutans	2.5mg	16±0.0
		1.5mg	-

Table 2: Determination of Minimum Inhibitory concentration of Standard (Ciprofloxacin) against S.mutans.

Standard Conc. (µg/well)	S.mutans	
	OD	% Inhibition
0	0.62	0
0.125	0.51	17.42
0.25	0.45	26.94
0.5	0.31	50
1	0.24	60.81
2	0.18	71.13
4	0.14	77.14
8	0.09	85.48
MIC(µg/well)	0.5 µg	

Table 3: Determination of Minimum Inhibitory concentration of raisin extract against S.mutans

Sample Conc. (µg/well)	S.mutans	
	OD	% Inhibition
0	0.60	0.00
15.62	0.51	15.19
31.25	0.43	28.61
62.5	0.38	37.39
125	0.31	48.65
250	0.26	57.10
500	0.20	67.37
1000	0.15	75.98
MIC (µg/well)	250µg	

5 | CONCLUSION

Raisins have been consumed for a long time and are commonly used in various food preparations. The studies have demonstrated an inverse association between intake of raisins and mortality from age related diseases such as coronary heart diseases. Anthocyanins, flavonoids and resveratrol are the major functional components that are responsible for most of biological activities of grape. The health benefits of grape are thought to arise mainly from bioactivities of their polyphenols.

Raisins contain polyphenols, antioxidants, flavonoids and iron that may benefit overall human health. Black raisins are found to have antibacterial efficacy against *S.mutans* and also has several other health benefits. It has an MIC value of 125 µg/ml at which the rate of inhibition is 48.65%. Raisins are worthy of further studies that may lead to the identification of new functional constituents due to their distinctive structure and marked biological activities of the compounds present in them.

Structural diversities and pronounced biological activities of compounds in raisins indicate that going green has a major benefit to the patients and also to environment thus the usage of raisins should be promoted in daily usage.

6 | ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The Authors declare no Conflict of interest.

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