IJBPAS, October, 2021, 10(10): 3419-3427

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

GRAPE (*VITIS VINIFERA* L.) PEEL EXTRACT: THE STUDY OF ANTIDIABETIC, ANTIBACTERIAL ACTIVITIES AND GC-MS ANALYSIS

PRIYADHARSHINI R[1], JUSTIN PACKIA JACOB[1], SIVARAJ C[2*], ARUMUGAM P[2] AND VENKATESAN P[3]

1: Department of Biotechnology, St. Joseph's College of Engineering, Sholinganallur, Chennai-600119, Tamilnadu, India

2: Phytochemistry and Natural products Research Laboratory, Armats Biotek Training and Research Institute, Guindy, Chennai-600032, Tamilnadu, India

3: Department of Chemistry, Thangavelu Engineering College, Chennai- 600096, Tamilnadu, India

***Corresponding Author: Dr. C. Sivaraj: E Mail: shivaraj27@gmail.com; Contact Number: +91-9941764053**

Received 21st Nov. 2020; Revised 27th Dec. 2020; Accepted 5th Jan. 2021; Available online 1st Oct. 2021

https://doi.org/10.31032/IJBPAS/2021/10.10.5643

ABSTRACT

Vitis vinifera commonly called Grape is a deciduous woody climber with coiled climbing tendrils and large leaves. It has small, pale, green flowers in the summer followed by bunches of berry fruits that range from green to purple-black. The leaves of the plant, which have astringent and hemostatic properties, are used in the treatment of diarrhea, hemorrhage, varicose veins, hemorrhoids, an inflammatory disorder, pain, hepatitis, and free radical related diseases. The objective of the study was to evaluate antidiabetic, antibacterial activities, and GC-MS analysis of methanol Grape peel extract. The maximum α-amylase enzyme inhibition was 88.17±0.91% at 120 µg/mL concentration and the IC_{50} was 14.33 µg/mL concentration. The antibacterial activity of Grape peel extract showed the zone of inhibition of 22 mm against *Staphylococcus aureus* at 500 µg/mL concentration. The GC-MS analysis showed the presence of antibacterial compounds such as 14,17- Octadecadienoic acid, methyl ester, Hexadecanoic acid methyl ester, 2-Acetyl-3,5,8-trihydroxy-6-methoxy-1,4-Naphthoquinone in the methanol Grape peel extract.

Keywords: *Vitis vinifera*, antioxidant activity, DPPH, antibacterial, antidiabetic, GC-MS

INTRODUCTION

Grapes which is *Vitis vinifera* is one of the fruit crops grown widely in many areas of the world. Grapes belong to the family Vitaceae, cultivated originally in the Asia Mediterranean region and southern Europe **[1]**. The fruit is a berry and there are between 5,000 and 10,000 varieties of grapes, though only a few are of commercial significance for wine and table grape production **[2]**. It is a great source of phenolic compounds. There are many classes of negatively charged polyphenols have been identified in grapes, such as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives (resveratrol), flavan-3-ols (catechin, epicatechin), flavonols (kaempferol, quercetin, myricetin), anthocyanins, etc. These polyphenols possess many beneficial effects on human health such as inhibition of free radical damage, antibacterial, antifungal, decreasing the risk of cardiovascular diseases as well as anticarcinogenic and anti-inflammatory activities **[3]**.

Taxonomy

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiosperms

Class: Eudicots

Order: Vitales

Family: Vitaceae

Genus: Vitis

Species: vinifera

Binomial name: *Vitis vinifera*

Figure 1: ***Vitis vinifera***

MATERIALS AND METHODS

Collection of peels and preparation of extract

The grapes were purchased at Royapuram fruit market, Chennai, Tamilnadu, India. The peels were removed washed and shade dried for 1 day. Then about 5 g of dried peel powder was soaked in methanol for 72 h. The reddish-orange supernatant liquid was filtered by filter paper and condensed

in a rotary evaporator at 50°C, which yields gummy extract.

Antidiabetic activity

Alpha-amylase enzyme inhibition

α- Amylase enzyme inhibition assay was carried out based on the starch-iodine test **[4]**. The assay mixture consists of various concentrations (20-120 µg/mL) of Grape peel extract, 10 µL of alpha-amylase enzyme prepared in 0.02 M sodium phosphate buffer saline (pH 6.9 containing 6 mM sodium chloride) and incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction mixture and incubated at 37°C for 60 min. One hundred µL of 1 M HCl was added to stop the enzymatic reaction and followed by 200 µL of iodine reagent (5 mM I_2 and 5 mM KI) was added. The color change was noted and the absorbance was read at 595 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. A dark-blue color indicates the presence of starch; yellow color indicates the absence of starch, while a brownish color indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark-blue color complex, whereas no color complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase **[5]**.

Antibacterial activity

Microbial strains

The microorganisms of Gram-positive strains such as *Bacillus subtilis, Staphylococcus aureus*, and *Micrococcus luteus* as well as Gram-negative strains such as *Escherichia coli* were used for the evaluation of the antibacterial activity. The aseptic chamber which consists of a wooden box (1.3m x 1.6m x 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short-wave UV light (from the lamp).

Nutrient broth agar medium

Nutrient broth agar medium was prepared (peptone-5 g; yeast extract-3 g; NaCl-5 g; distilled water-1000 mL; pH-7.0±0.2; Agar-20 g) according to the standard method **[6]**. The required amount of reagents was weighed, suspended in the required volume of 200 mL distilled water in a 500 mL conical flask, stirred and then autoclaved at 15 lbs and 121ºC for 15 min. The hot medium was poured in sterile petri plates which were kept in the aseptic Laminar chamber. The medium was allowed to solidify for 15 min **[7]**.

Agar well-diffusion method

The antibacterial activity of Grape peel extract was analyzed by the agar well diffusion method **[8]**. The solidified

nutrient agar in the petri plates was inoculated by dispensing the inoculums by sterilized cotton swabs and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The extract was then poured into each well to get desirable concentrations. All the plates containing sample loaded wells were incubated at 37°C for 24 h. The zone of inhibition in each concentration of extract and standard was measured in each plate by calculating the diameter of the zone of inhibition **[9]**. Tetracycline was used as the standard for bacteria. The controls consist of solidifying agar onto which was solvent, and the extract was soluble in it.

Gas chromatography–Mass Spectrometry (GC–MS)

The Grape peel extract was injected into an HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. The chromatographic conditions were used as helium as the carrier gas; the flow rate of 1 mL/min; the injector was operated at 200°C and the column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. The mass conditions were used as ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C and the mass range of 50-600 mass units. The database of the National Institute of Standards and Technology (NIST) was used for the interpretation of the mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library **[10]**.

RESULTS AND DISCUSSION

Antidiabetic activity

Alpha-amylase enzyme inhibition

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and type II is the major form of diabetes. Many herbal products have been reported to have anti-diabetic activities and are used for the treatment of diabetes in Ayurveda. Herbal extracts have been unavoidable ingredients in modern medicines and active compounds from herbal plants are used directly or indirectly for the preparation of allopathy medicines. The maximum α-amylase enzyme inhibition of Grape peel extract was 88.17±0.91% at 120 µg/mL concentration and the IC_{50} was 14.33 µg/mL concentration. The result showed that it may lower the blood glucose level, which may be due to the increased level of insulin in the blood **[11]**. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels. Synthetic enzyme inhibitors of diabetes are

known to have gastrointestinal side effects and inhibitors from natural sources having fewer side effects **[12]**.

Antibacterial activity by agar well diffusion method

The antibacterial activity was carried out for the Grape peel extract against Gram-positive bacteria such as Bacillus subtilis, Staphylococcus aureus, and Micrococcus luteus and Gram-negative bacteria such as Escherichia coli. The antibacterial activity of the Grape peel extract was measured by the diameter of the clear zone around the well in petri plates. The antibacterial activity of the peel extract may be the presence of secondary metabolites such as terpenoids, and phenolic compounds that adversely affect the growth and metabolism of microbes **[13]**. The antibacterial activity of Grape peel extract showed the zone of inhibition of 22 mm against Staphylococcus aureus at 500 µg/mL concentration. Most of the pathogenic bacteria are intrinsically susceptible to at least one of the secondary metabolites. They are bactericidal agents that kill bacteria by interrupting peptidoglycan (cell wall) biosynthesis. Their targets are penicillin-binding proteins (PBPs) that exhibit transglycosylase transpeptidase or carboxypeptidase activities in both Gram-positive and Gram-negative bacteria **[14]**.

GC-MS analysis

GC-MS analysis was carried out for the Grape peel extract of *Vitis vinifera* and the antibacterial compounds such as 14,17-Octadecadienoic acid, methyl ester, Hexadecanoic acid, methyl ester **[15]**, 2-acetyl-3,5,8-trihydroxy-6-methoxy-1,4-Naphthoquinone **[16]** were eluted and recorded.

Table 1: α-Amylase enzyme inhibition activity of Grape peel extract

S. No.	Concentration (µg/mL)	α-Amylase inhibition at 595 nm
		% of inhibition
1	20	69.76±1.22
2	40	74.97±0.16
3	60	76.96±0.46
4	80	84.03±0.65
5	100	85.75±0.72
6	120	88.17±0.91

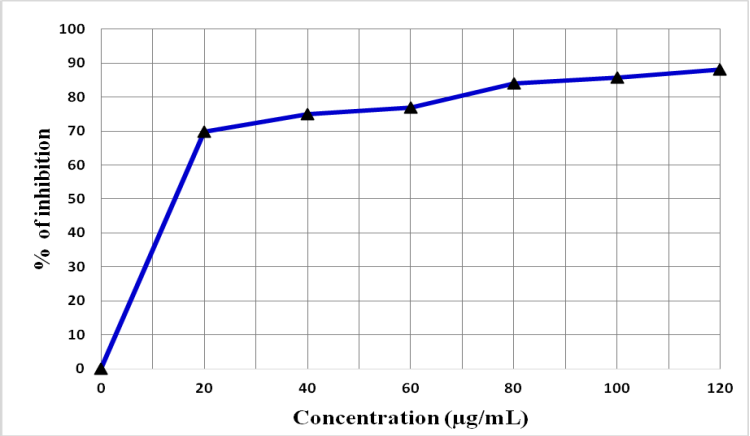


Figure 2: α-Amylase enzyme inhibition activity of Grape peel extract

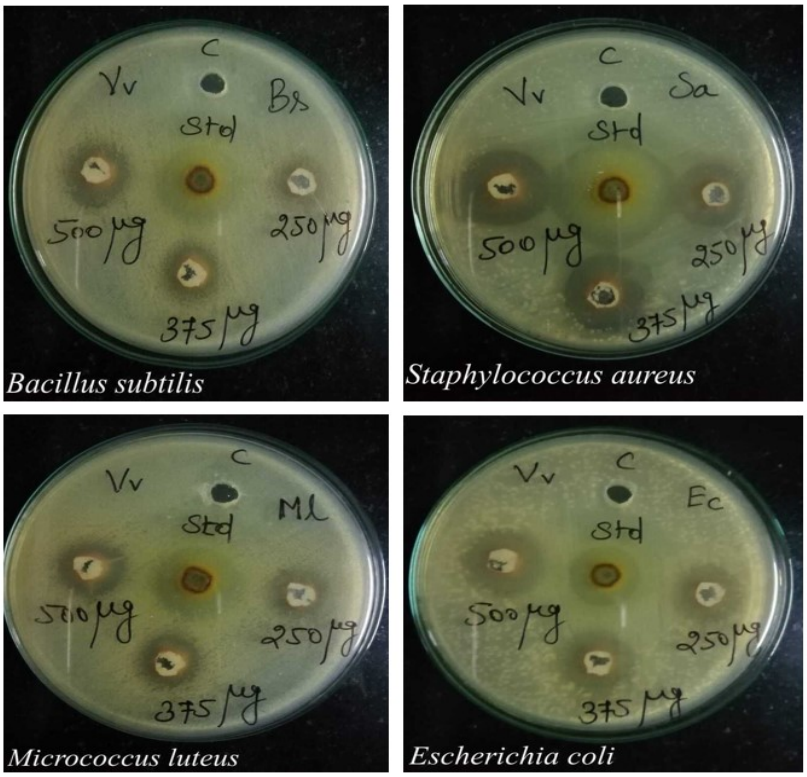
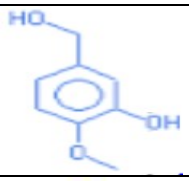
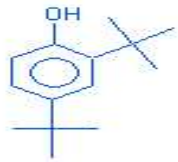
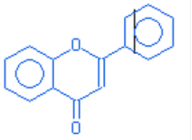
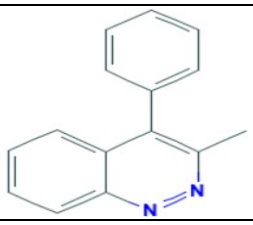
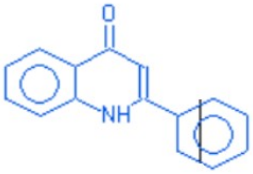
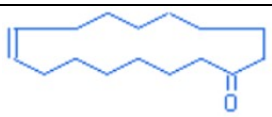
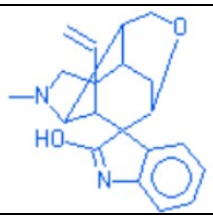


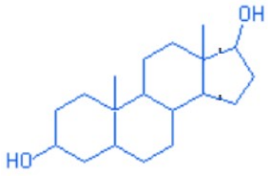
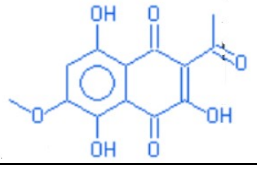
Figure 3: Antibacterial activity of Grape peel extract

Table 2: Antibacterial activity of Grape peel extract

Bacterial pathogens	Zone of inhibition (mm)			
	250 µg/mL	375 µg/mL	500 µg/mL	Standard (Tetracycline)
Bacillus subtilus	12	14	16	18
Staphylococcus aureus	20	20	22	24
Micrococcus luteus	17	18	21	16
Escherichia coli	16	18	20	22

Table 3: GC-MS analysis of Grape peel extract

S. No	RT	Name	Structure	Mol. Wt g/mol	Mol. formula
1.	10.22	3-Hydroxy-4-methoxybenzyl alcohol		154.16	$C_8H_{10}O_3$
2.	10.73	Phenol-2,4 bis(1,1-dimethylethyl)		196.01	$C_{22}H_{30}O$
3.	17.78	1-Docosene		308.6	$C_{22}H_{44}$
4.	13.07	Flavone[10][11]		222.000	$C_{15}H_{10}O_2$
5.	15.22	Hexadecanoic acid, methyl ester		270.45	$C_{17}H_{34}O_2$
6.	11.53	3-methyl-4-phenyl cinnoline		220.27	$C_{15}H_{12}N_2$
7.	12.32	4[1H]-Quinolinone,2-phenyl		223.27	$C_{15}H_{13}NO$
8.	14.95	8-Cyclohexadecen-1-one		236.39	$C_{16}H_{28}O$
9.	16.98	14,17-Octadecadienoic acid, methyl ester		294.5	$C_{19}H_{34}O_2$
10.	13.77	Cyclohexadecane		224.42	$C_{16}H_{32}$
11.	22	Gelsemine		322.40	$C_{20}H_{22}N_2O_2$

12.	**15.43**	**Hombreol**		**292.00**	$C_{19}H_{32}O_2$
13.	**15.85**	**2-acetyl-3,5,8-trihydroxy-6-methoxy-1,4-Naphthoquinone**		**278.00**	$C_{13}H_{10}O_7$

CONCLUSION

Plants signify an inexhaustible source of novel molecules for recent drug discovery. Herbal plant extracts and their active constituents have long been used in traditional medicine for the treatment of several diseases. The appearance of antibiotic-resistant microbial strains and the rebirth of newer and more deadly diseases have called for the need to discover novel antibiotics. The whole natural resources are being screened to identify potential drug leads and recent advances in the development of techniques for isolation, characterization and pharmacological evaluation have led to an interest in plant secondary metabolites as a source of new drugs. Natural product research is continuously exploring the chemical diversity of several lead molecules, which can be used as templates for new drug discovery. The study of antimicrobial activity showed that Grape peel extract could be used for the development of novel antimicrobial agents.

ACKNOWLEDGEMENT

The authors are thankful to IIT Madras for helping GCMS analysis

REFERENCES

[1] Elagamey. A. A.; *et al*.; Comparative study of morphological characteristics and chemical constituents for seeds of some grape table varieties, *Journal of American Science*, 9(1), **2013**, 447-454.

[2] Robinson. J.; "Wine & Spirits Education Trust" Wine and Spirits: Understanding Wine Quality, **2011**, 2-5.

[3] Yadav. D.; Kumar. A.; Kumar. P.; Mishra. D.; Antimicrobial properties of black grape (*Vitis vinifera* L.) peel extracts against antibiotic-resistant pathogenic bacteria and toxin producing molds, *Indian journal of pharmacology*, 47(6), **2015**, 663.

[4] Hossain, S. J.; El-Sayed. M.; and Aoshima. H.; Antioxidative and anti-α-amylase activities of four wild plants consumed by pastoral nomads in Egypt, *Orient Pharm Exp Med*, 9(3), **2009**.

[5] Hansawasdi. C.; Kawabata. J.; Kasai. T.; Alpha amylase inhibitors from Roselle (*Hibiscus sabdariffa* Linn.) tea,

Bioscience, Biotechnology and Biochemistry, 64(5), **2000**, 1041-1043.

[6] Usman. J. G.; Sodipo. O. A.; Sandabe. U. K.; In vitro antimicrobial activity of *Cucumis metuliferus* E. Mey. Ex. Naudin fruit extracts against *Salmonella gallinarum, Int J Phytomed*, 6(2), **2014**, 268–274

[7] Abalaka. M. E.; Daniyan. S. Y.; Oyeleke. S. B.; Adeyemo. S. O.; The antibacterial evaluation of *Moringa oleifera* leaf extract on selected bacterial pathogens, *J Microbiol Res*, 2(2), **2012**, 1–4.

[8] Balouiri. M.; Sadiki. M.; Ibnsouda. S. K.; Methods for in vitro evaluating antimicrobial activity: A review, *J Pharm Anal*, 6(2), **2016**, 71-79.

[9] Valgas. C.; Souza. S. M. D.; Smania. E. F.; Screening methods to determine antibacterial activity of natural products, *Braz. J. Microbiol*, 38(2), **2007**, 369–380.

[10] Ravisankar. N.; Sivaraj. C.; Seeni. S.; Jerrine joseph; Raaman. N.; GC-MS Analysis and anticancer activity of methanol extract of leaves of *Hypericum hookerianum* wight & arn, *Int J Pharm Pharm Sci*, 6(5), **2014**, 515-519.

[11] McCue. P.; Vattem. D.; Shetty. K.; Inhibitory effect of clonal oregano extracts against porcine pancreatic amylase in vitro, *Asia Pacific Journal of Clinical Nutrition*, 13(4), **2004**, 401–408.

[12] Ali. H.; Houghton. P. J.; Amala. S.; α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*, *Journal of Ethnopharmacology*, 107(3), **2006**, 449–455.

[13] Jayaprakasha, G. K.; Tamil Selvi.; Sakariah. K. K.; Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts, *Food research international*, 36(2), **2003**, 117-122.

[14] Al-Salt. J.; Antimicrobial activity of crude extracts of some plant leaves, *Res. J. Microbiol*, 7 (1), **2012**, 59-67.

[15] Rahman, M. M.; Ahmad, S. H.; Mohamed, M. T.; Ab Rahman, M. Z.; Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and Andrographis paniculata.; *Scientific World Journal* 2014, 2014:635240.

[16] Sánchez-Calvo. J. M.; Barbero. G. R.; Guerrero-Vásquez. G. *et al*.; Synthesis, antibacterial and antifungal activities of naphthoquinone derivatives: a structure–activity relationship study, *Medicinal Chemistry Research*, 25(6), **2016**, 1274–1285.