The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

A grape (*Vitis vinifera* L.) pomace water extract: phenolic composition and protective effects in the colon

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Annalisa Chiavaroli [1,*], Lucia Recinella[1], Serena Veschi[1], Alessandro Cama[1], Alessandra Acquaviva[1,2], Maria Loreta Libero[1], Sheila Leone[1], Simonetta Cristina Di Simone[1], Ester Pagano[3], Gokhan Zengin[4], Luigi Menghini[1], Luigi Brunetti[1], Angelo Antonio Izzo[3], Giustino Orlando[1], Claudio Ferrante[1]

[1] Department of Pharmacy, G. d'Annunzio University, Chieti-Pescara, Italy
[2]Veridia Italia Srl, Pescara, Italy
[3]Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy
[4]Physiology and Biochemistry Laboratory, Department of Biology, Science Faculty, Selcuk University, Konya, Turkey
* Corresponding author: annalisa.chiavaroli@unich.it

veridia

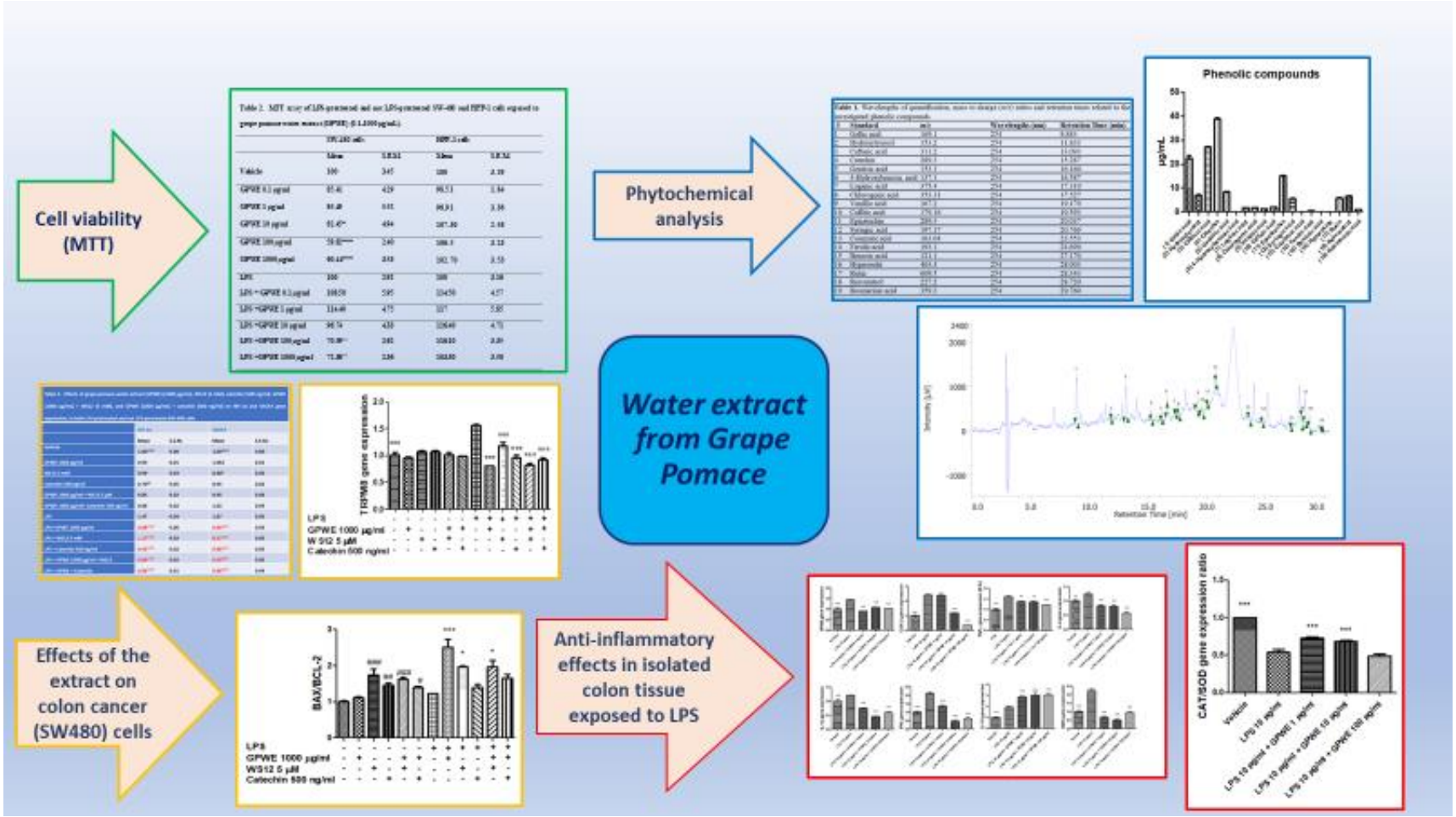
Ud'A Università degli Studi "G. d'Annunzio"

DF DIPARTIMENTO DI FARMACIA

SELÇUK ÜNİVERSİTESİ

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II - DIPARTIMENTO DI FARMACIA
DIPARTIMENTO DI ECCELLENZA 2018-2022 AREA "03 - SCIENZE CHIMICHE"

A grape (*Vitis vinifera* L.) pomace water extract: phenolic composition and protective effects in the colon



ECMC 2022
The 8th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2022 | ONLINE

Abstract:
Grape (*Vitis vinifera* L.) pomace is a residue derived from the winemaking process, which contains bioactive compounds displaying noteworthy health-promoting properties. A characterization of the water extract from grape pomace from Montepulciano d'Abruzzo variety (Villamagna doc) was conducted, and the bioactive phenolic compounds were quantified through HPLC-DAD-MS analysis. The aim of the present study was to investigate the phenolic composition and protective effects of a water extract of grape pomace (WEGP) in colorectal cancer cell line SW480 and in isolated mouse colon exposed to Escherichia coli lipopolysaccharide (LPS). The extract decreased SW-480 cell viability, as well as vascular endothelial factor A (VEGFA), hypoxia-induced factor 1α (HIF1α), and transient receptor potential M8 (TRPM8) LPS-induced gene expression. Moreover, the extract inhibited mRNA levels of nuclear factor kB (NFkB), cyclooxygenase (COX)-2, tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-1β, IL-10, inducible nitric oxide synthase (iNOS), and interferon (IFN)γ, in isolated colon. Conversely, WEGP increased the gene expression of antioxidant catalase (CAT) and superoxide dismutase (SOD), in the same model. The modulatory effects exerted by WEGP could be related, at least in part, to the phenolic composition, with particular regards to the catechin level. Grape pomace could be considered as a valuable source of bioactive extracts and phytochemicals with protective effects in the colon.

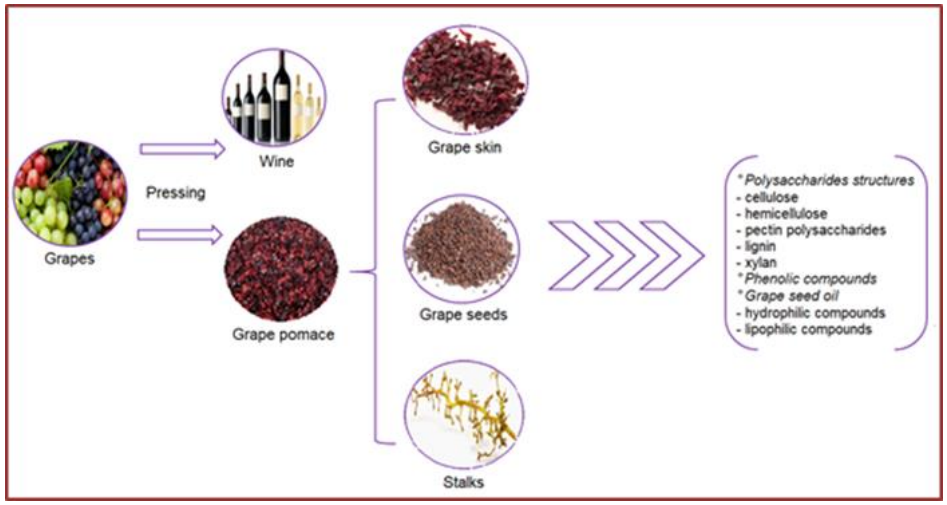
Keywords: Catechin, Colon cancer, Grape pomace, Inflammation, TRPM8, Vitis vinifera

Introduction

- Grape pomace is one of the main solid by-products deriving from the winemaking process, which contains seeds, skin residues, and stems[1]
- It contains neutral polysaccharides (30%), pectic substances (20%), insoluble proanthocyanidins (15%), and phenolic compounds, which mainly include resveratrol, anthocyanins, flavones, and tannins[2]
- A wide body of evidence hinted some biological activities exerted by grape polyphenols, including antioxidant, cardioprotective, anticancer, anti-inflammatory, antiaging, and antimicrobial properties[3, 4]

1. Antonic´, B.; Janc˘íková, S.; Dordevic´, D.; Tremlová, B. Grape Pomace Valorization: A Systematic Review and Meta-Analysis. Foods 2020, 9, 1627.
2. Spinei, M., & Oroian, M. (2021). The potential of grape pomace varieties as a dietary source of pectic substances. Foods, 10(4), 867.
3. De la Cerda-Carrasco, A.; López-Solís, R.; Nuñez-Kalasic, H.; Peña-Neira, A.; Obreqeu-Slier, E. Phenolic composition and antioxidant capacity of pomaces from four grape varieties (Vitis vinifera L.). J. Sci. Food Agric. 2015, 95, 1521–1527.
4. Nassiri-Asl, M.; Hosseinzadeh, H. Review of the Pharmacological Effects of Vitis vinifera (Grape) and its Bioactive Constituents: An Update. Phytother. Res. 2016, 9, 1392–1403.

Introduction



M. Spinei and M. Oroian: The Potential of Grape Pomace Varieties as a Dietary Source of Pectic Substances. Foods 2021, 10(4), 867.

Parts of grape bunch	Major phytochemicals
Peel 	• Anthocyanins • Quercetin derivatives (e.g. rutin) • Kaempferol derivatives • Catechins • Resveratrol • Chlorogenic
Seed 	• Catechins • Chlorogenic acid • Quercetin derivatives • Resveratrol
Stem 	• Caftaric acid, coutaric acid • Quercetin derivatives • Kaempferol derivatives • Myricetin derivatives • Catechins • Astilbin, engeletin

(Rockenbach et al., 2011; Souquet et al., 2000)

Introduction

- In addition, the health-promoting effects induced by the phenolic compounds of red wine are well known
- Many pharmacological activities were ascribed to different parts of grapes [5]
- Grape seed extract and its constituents were found to exert protective effects on TNBSS- or DSS-induced ulcerative colitis in rodents[6]
- Moreover, dietary grape seed extract supplementation has been previously considered to exert preventive effects against colorectal carcinogenesis[7]

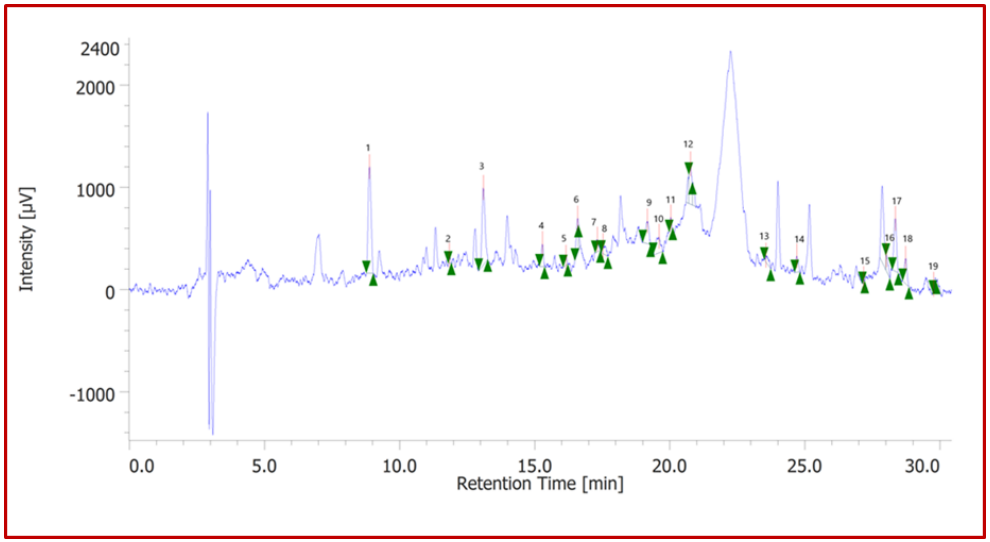
5. Nassiri-Asl, M.; Hosseinzadeh, H. Review of the Pharmacological Effects of Vitis vinifera (Grape) and its Bioactive Constituents: An Update. Phytother. Res. 2016, 9, 1392–1403.
6. Cheah, K. Y., Bastian, S. E., Acott, T., Abimosleh, S. M., Lymn, K. A., & Howarth, G. S. (2013). Grape seed extract reduces the severity of selected disease markers in the proximal colon of dextran sulphate sodium-induced colitis in rats. Digestive diseases and sciences, 58(4), 970-977.
7. Tian, Q., Xu, Z., Sun, X., Deavila, J., Du, M., & Zhu, M. (2020). Grape pomace inhibits colon carcinogenesis by suppressing cell proliferation and inducing epigenetic modifications. The Journal of Nutritional Biochemistry, 84, 108443.

Introduction

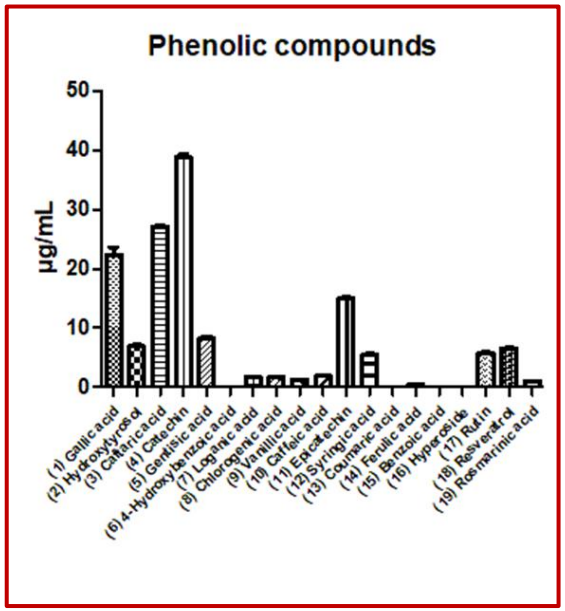
- In the present study, we aimed to evaluate the effects of a water extract of grape pomace (GPWE) in colorectal cancer cell line SW480 and in an ex vivo experimental model of colon inflammation constituted by isolated mouse colon challenged with *Escherichia coli* lipopolysaccharide (LPS)[8]
- The grape pomace was the by-product of the vintage (year 2020) of a DOC red wine, the Montepulciano d'Abruzzo variety (Villamagna doc)

8. Menghini, L., Ferrante, C., Leporini, L., Recinella, L., Chiavaroli, A., Leone, S., … Brunetti, L. (2016). An Hydroalcoholic chamomile extract modulates inflammatory and immune response in HT29 cells and isolated rat Colon. Phytotherapy Research, 30(9), 1513–1518.

Results and discussion: Characterization of the extract



Chromatographic analysis of the water extract from the *Vitis vinifera* pomace. The chromatographic analysis confirmed the presence of 19 phytochemicals. The prominent compound was catechin (peak #4). The other main phytochemicals were gallic acid (peak #1), caftaric acid (peak #3), and epicatechin (peak #11).



Phenolic profile of the grape pomace sample. Gallic acid, caftaric acid, and catechin were the main phytochemicals.

- 19 phytochemicals were identified through HPLC-DAD-MS, in comparison with pure standards
- Catechin was found to be the prominent flavonoid
- This is also consistent with the phytochemical profile of the water extract from the grape pomace collected during the 2019 vintage.

Effects of the extract on colon cancer (SW480) and fibroblast (HFF-1) cells: cell viability

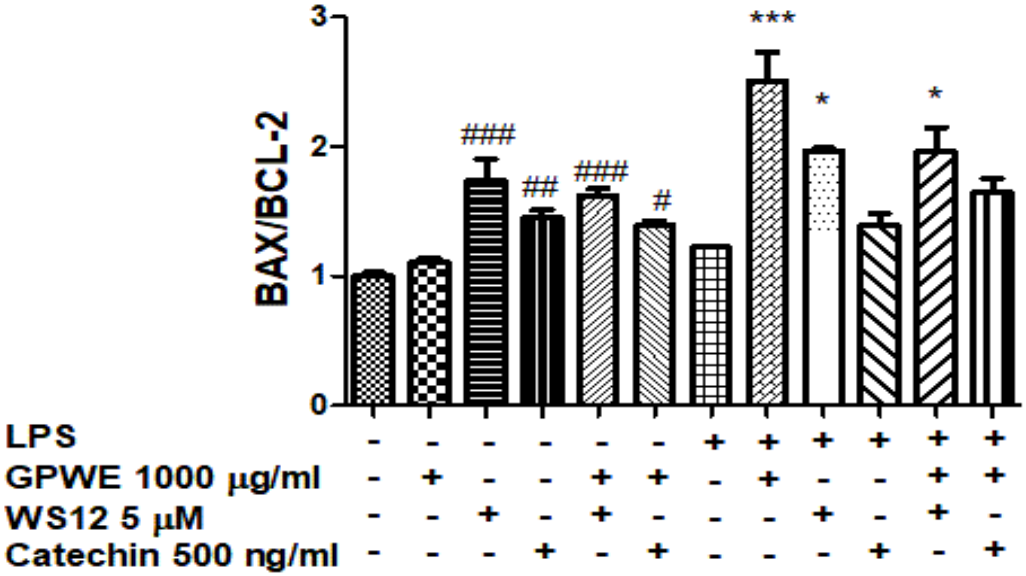
Table 2. MTT assay of LPS-pretreated and not LPS-pretreated SW-480 and HFF-1 cells exposed to grape pomace water extract (GPWE) (0.1-1000 µg/mL).

	SW-480 cells		HFF-1 cells	
	Mean	S.E.M.	Mean	S.E.M.
Vehicle	100	3.45	100	3.29
GPWE 0.1 µg/ml	85.41	4.29	98.51	1.84
GPWE 1 µg/ml	85.49	5.52	96.91	3.36
GPWE 10 µg/ml	82.45*	4.94	107.30	2.58
GPWE 100 µg/ml	59.82***	2.40	106.5	3.23
GPWE 1000 µg/ml	60.44***	3.53	102.70	3.53
LPS	100	2.92	100	3.16
LPS + GPWE 0.1 µg/ml	108.50	5.95	114.50	4.57
LPS +GPWE 1 µg/ml	114.40	4.75	117	5.85
LPS +GPWE 10 µg/ml	96.74	4.33	116.40	4.71
LPS +GPWE 100 µg/ml	70.59##	2.62	116.10	3.35
LPS +GPWE 1000 µg/ml	72.36##	2.36	103.30	3.58

MTT assay of LPS-pretreated and not LPS-pretreated SW-480 and HFF-1 cells exposed to grape pomace water extract (GPWE) (0.1–1,000 µg/ml)Data are reported as means ± S.E.M. ANOVA, $P < 0.0001$; *$P < 0.05$, ***$P < 0.001$ vs. vehicle; #$P < 0.001$ vs. LPS.

- The water grape pomace extract was able to induce a significant reduction of SW-480 cell viability in both basal and LPS-induced inflammatory conditions
- The effects were relevant starting from the concentrations of 100 µg/ml, at which the cell viability was under the limits of biocompatibility
- By contrast, the HFF-1 cell line viability was not modified by the extract, thus, excluding any cytotoxic effect in nontumoral cells.

Potential pro-apoptotic effects of the extract



Effects of grape pomace water extract (GPWE) (1000 µg/mL), WS12 (5 mM), catechin (500 ng/ml), GPWE (1000 µg/mL) + WS12 (5 mM), and GPWE (1000 µg/mL) + catechin (500 ng/ml) on BAX/BCL-2 gene expression ratio, in both LPS-pretreated and not LPS-pretreated SW-480 cells. Data are reported as means ± SEM. ANOVA, $P < 0.001$; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ vs. Vehicle; $^{***}P<0.001$, $^{*}P < 0.05$ vs. LPS.

- BAX and BCL-2 are well known to play opposite effects on apoptosis, with BAX and BCL-2 displaying proapoptotic and anti-apoptotic effects, respectively
- BAX/BCL-2 ratio represents a reliable index a proapoptotic activity
- The increase in BAX/BCL-2 gene expression ratio following GPWE treatment could underlie a possible influence of the extract on apoptosis pathway, in SW-480 cells

Inhibitory effects on LPS-induced increase in HIF-1α and VEGFA gene expression

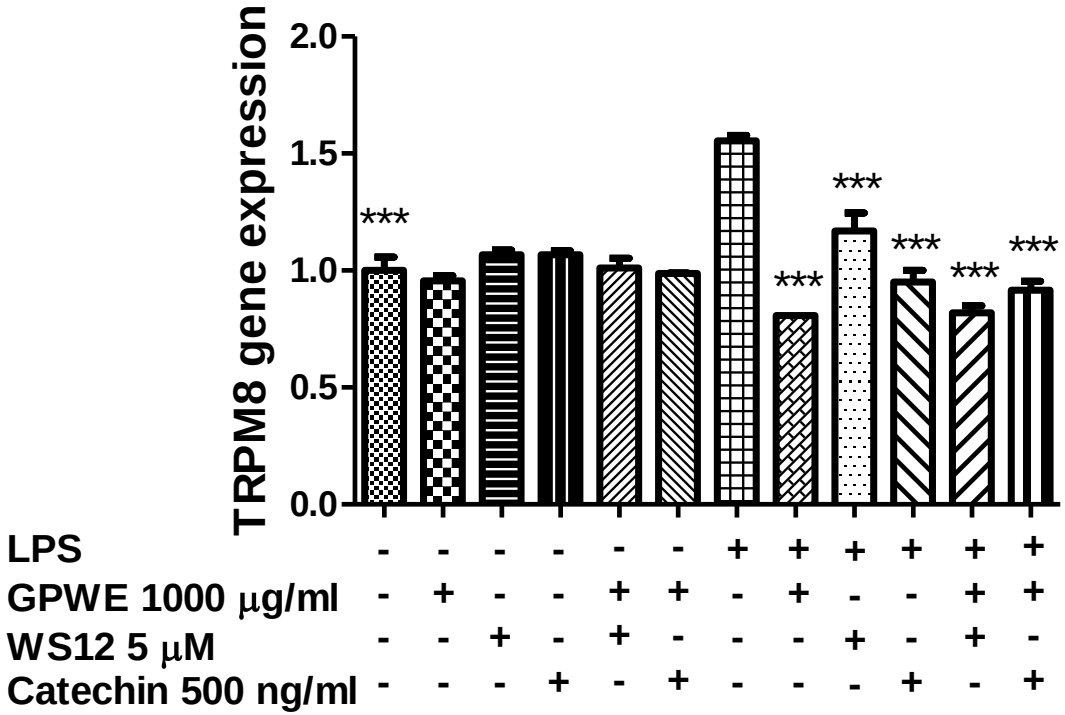
Table 3. Effects of grape pomace water extract (GPWE) (1000 µg/mL), WS12 (5 mM), catechin (500 ng/ml), GPWE (1000 µg/mL) + WS12 (5 mM), and GPWE (1000 µg/mL) + catechin (500 ng/ml) on HIF-1α and VEGFA gene expression, in both LPS-pretreated and not LPS-pretreated SW-480 cells

	HIF-1α		VEGFA	
	Mean	S.E.M.	Mean	S.E.M.
Vehicle	1.00***	0.00	1.00***	0.00
GPWE 1000 µg/ml	0.93	0.01	1.081	0.01
WS12 5 mM	0.99	0.03	0.80$^{\#}$	0.03
Catechin 500 ng/ml	0.76$^{\#\#}$	0.05	0.95	0.02
GPWE 1000 µg/ml + WS12 5 µM	0.85	0.02	0.95	0.06
GPWE 1000 µg/ml+ Catechin 500 ng/ml	0.86	0.02	1.02	0.04
LPS	1.47	0.04	1.67	0.05
LPS+ GPWE 1000 µg/ml	0.88***	0.06	0.80***	0.03
LPS + WS12 5 mM	1.17***	0.02	0.57***	0.05
LPS + Catechin 500 ng/ml	0.93***	0.02	0.88***	0.05
LPS + GPWE 1000 µg/ml + WS12	0.86***	0.02	0.69***	0.05
LPS + GPWE + Catechin	0.86***	0.01	0.88***	0.04

- Together with the HIF1α, VEGFA is a well-known angiogenesis stimulating factor, and is considered a key mediator of the so-called inflammatory to cancer transition
- The gene expression of both factors was downregulated by the extract, and the effect was more relevant when the cells were challenged with LPS

Effects of grape pomace water extract (GPWE) (1,000 µg/ml), WS12 (5 mM), catechin (500 ng/ml), GPWE (1,000 µg/ml) + WS12 (5 mM), and GPWE (1,000 µg/ml) + catechin (500 ng/ml) on HIF-1α and VEGFA gene expression, in both LPS-pretreated and not LPS-pretreated SW-480 cellsData are reported as means ± SEM. ANOVA, $P < 0.0001$; $^{\#}P<0.05$, $^{\#\#}P < 0.01$ vs. Vehicle, $^{***}P < 0.001$ vs. LPS.

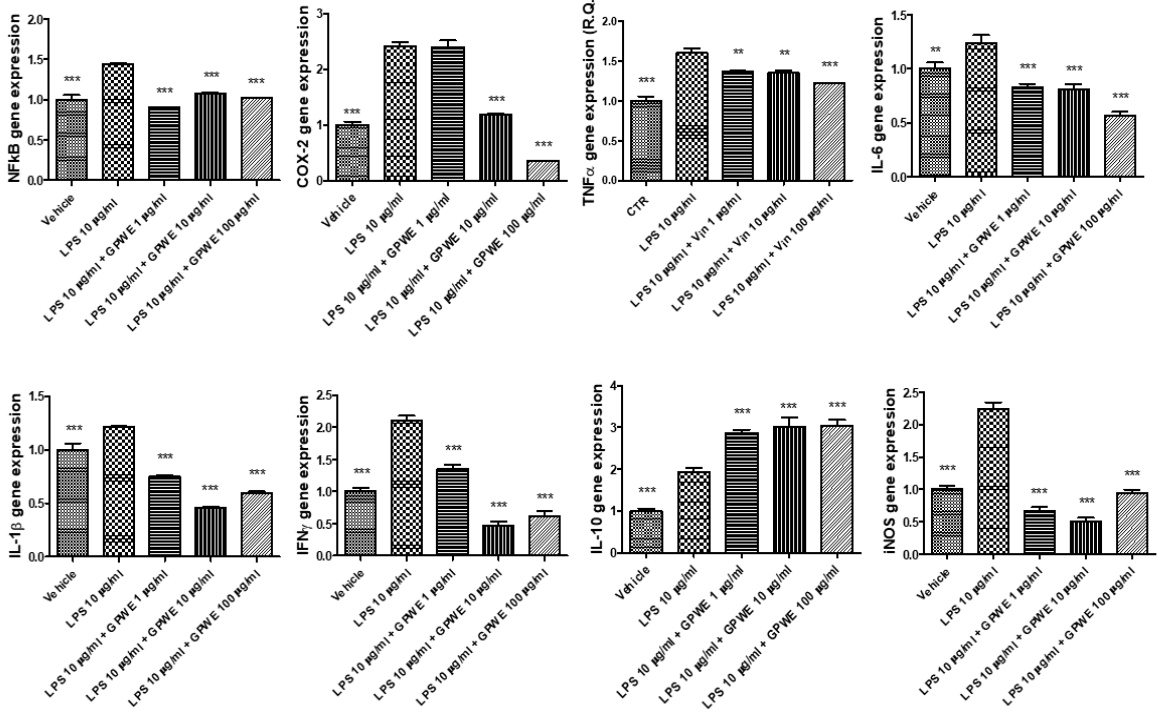
Inhibitory effects on LPS-induced increase in TRPM8 gene expression



Effects of grape pomace water extract (GPWE) (1000 μg/mL), WS12 (5 mM), catechin (500 ng/ml), GPWE (1000 μg/mL) + WS12 (5 mM), and GPWE (1000 μg/mL) + catechin (500 ng/ml) on TRPM8 gene expression, in both LPS-pretreated and not LPS-pretreated SW-480 cells. Data are reported as means ± SEM. ANOVA, $P < 0.0001$; ***$P < 0.001$ vs. LPS.

- In colon cancer, there is increasing evidence of the involvement of transient receptor potential (TRP) M8 (TRPM8), whereas its block has been related, albeit partially, to the inhibition of carcinogenesis, in mice
- In SW480 cells, the extract reduced the gene expression of TRPM8, and this was mirrored by the corresponding inhibition of TRPM8 gene expression by catechin
- WS12, a selective TRPM8 agonist, determined an analogue inhibition of the receptor gene expression

Anti-inflammatory effects in isolated colon tissue exposed to LPS

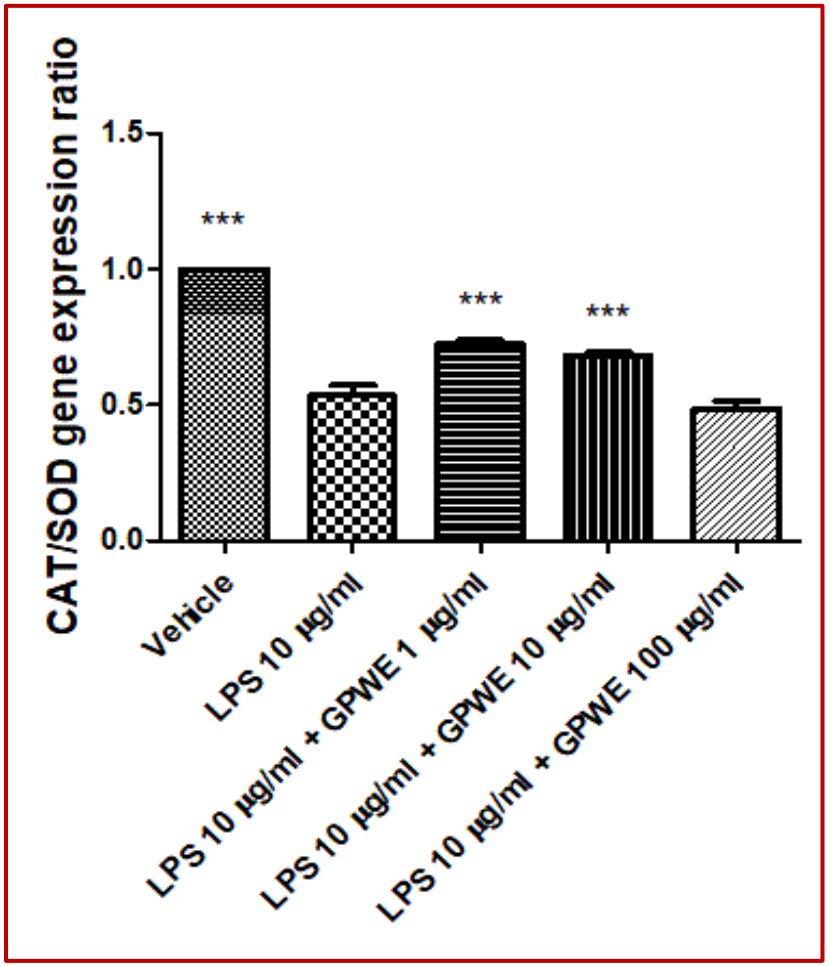


Effects of grape pomace water extract (GPWE) (1, 10, and 100 µg/mL) on NF-kB, COX-2, TNF-α, IL-6, IL-1β, IFNγ, IL-10, and iNOS gene expression, in mouse colon specimens. Data are reported as means ± SEM. ANOVA, $P < 0.0001$; **$P < 0.01$, ***$P < 0.001$ vs. LPS.

✓ The extract was also tested in an ex vivo experimental model of colon inflammation, constituted by isolated mouse colon specimens exposed to LPS

- The extract was effective in reducing the LPS-induced gene expression of different proinflammatory biomarkers involved in colon inflammation: nuclear factor kB (NFkB), cyclooxygenase (COX)-2, tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-1β, and interferon (IFN)γ
- In parallel, the extract was effective in stimulating the gene expression of the anti-inflammatory cytokine IL-10
- Besides, the gene expression of inducible nitric oxide synthase (iNOS), deeply involved in nitrosative stress, was inhibited

Inhibitory effects on LPS-induced increase in CAT/SOD gene expression



Effects of grape pomace water extract (GPWE) (1, 10, and 100 µg/mL) on CAT/SOD gene expression ratio, in mouse colon specimens. Data are reported as means ± SEM. ANOVA, $P < 0.0001$; ***$P < 0.001$ vs. LPS.

- The CAT/SOD gene expression ratio, index of antioxidant activity, was augmented by GPWE, but only at the lowest tested concentrations
- We cannot exclude that this could depend upon possible pro-oxidant effects induced by phenolic compounds, in solution

Conclusions

- The results of the study indicated the efficacy of the water extract from grape pomace in reverting the burden of inflammation and oxidative stress occurring in isolated colon specimens exposed to LPS
- Whereas, the reduction of human colon cancer SW-480 cell viability, and the modulation of pattern of gene expression of proteins involved in carcinogenesis, further support protective effects in the colon
- The mechanism underlying these effects could involve more than one phytochemical

Conclusions

- However, in SW480 cells the prominent phenolic compound, namely catechin, could be the main responsible of the inhibitory effects on VEGFA, HIF1α, and TRPM8 gene expression
- Considering also the large amount of grape pomace, about 20% of all processed plant material during wine-making the present study strongly suggests the use of the pomace as a high-quality by-product.
- This could also lead to an overall improvement of the chain production, and this would be of particular importance for the local native-ecotypes, in most cases showing high quality wines, such as the Villamagna DOC, but with low productivity

Acknowledgments

The present study is also part of the third mission activities of the botanical garden “Giardino dei Semplici” of “G. d’Annunzio” University.

Orto Botanico Officinale "Giardino dei Semplici"

Thanks for Your kind hosting and attention