Antiviral activity of medicinal plants extracts against foodborne norovirus

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Medicinal plant extracts have a broad antimicrobial activity, including antiviral effects. In our study, eleven dry extracts of ten different medicinal plants prepared with two solvents (5%, dimethyl sulfoxide (DMSO) and 30% ethanol in 5% DMSO) in different concentrations have been examined for anti-norovirus activity. The reduction of norovirus >1 $\log_{10}$ genome equivalents has been obtained with 1 mg/mL of *Aronia melanocarpa* leaf extract, dried wine Prokupac and *Hypericum perforatum* extract. Some of the observed extracts i.e. *Hypericum perforatum*, *Aronia melanocarpa* fruit extract and *Punica granatum* peel extract showed better activity when dissolved in 30% ethanol with 5% DMSO. All results of anti-noroviral activity of tested extracts which achieved <0.5 $\log_{10}$ genome equivalents were considered as not effective.

Key words: *Human norovirus, medicinal plant extract, antiviral activity*

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1. INTRODUCTION

Generally, human norovirus (hNoV) is recognized as one of the most common causes of viral gastroenteritis. Norovirus is easily transmitted from person to person by a fecal-oral route directly or indirectly through contaminated food (shells, berries, etc) or water. Also, hNoV are very often related with fresh products and large outbreaks are well documented (EFSA/ECDC, 2016). The European Union (EU) Rapid Alert System for Food and Feed (RASFF) indicated that norovirus is a relatively "new acquaintance" in terms of citation history in RASFF border rejection notifications (involving Third Countries as countries of origin) but a rather "old acquaintance" in alert notifications. The relative frequency of citation of alert notifications on norovirus places them well ahead of *Salmonella* and *Listeria*, well known bacterial food pathogens, and marks those foodborne viruses as a serious biological hazard (Papapanagiotou, 2017).

Norovirus belongs to the *Caliciviridae* family of non-enveloped, small, positive-stranded RNA viruses and based on their capsid amino acid compositions are divided into 6 genogroups (GI - GVI) and > 40 genotypes (Rocha-Pereira et al., 2016). HNoV as non-enveloped viruses are resistant to the majority of chemical disinfectants. One of the agents with virucidal efficacy is chlorine/hypochlorite, however due to its byproducts represents health risk (El-Senousy et al., 2014).

Numerous plants have been used in traditional medicine for healing but also for the preservation of raw and processed food (Jaykus et al., 2013; D'Souza, 2014). Plant extracts abundant in chemically diverse metabolites have been extensively studied for their varied biological activity including antimicrobial (Braga et al., 2005; D'Souza, 2014). Plant derived antimicrobials for the control and prevention of the transmission of human enteric viruses, including noroviruses, are of recent interest especially because of the absence of vaccines for the human noroviruses. The aim of our study was screening of dry extracts of ten different medicinal plants for anti-norovirus activity.

2. MATERIALS AND METHODS

2.1. hNoV stock

Fecal stool samples containing hNoV were diluted (w/v) to obtain 10% suspensions with phosphate buffered saline (PBS, pH=7.2). Suspensions were centrifuged (Eppendorf 5804 R, 14000 G / 5 min at 20 °C) and filtered using syringes (0.45 and 0.22 µm filter pore size; Thermo Fisher Scientific, Germany) in order to remove impurities. Target hNoV RNA (expressed as amount of genome equivalents; GE) in the suspension was quantified by standard curve which was prepared using positive control (PC) template (Quantification of Norovirus genotype 1 and 2, Primer DesignTM Ltd, genesig). The all further experiments were carried out with the 1% suspension of hNoV which contained $\approx 10^5$ GE per µL.

Table 1. Medicinal plants used for the testing of antiviral activity

Extracts	Abbreviation	Plant part
Sideritis raeseri	SRE	Herba
Gentiana asclepiadea	GAE	Root
Aronia melanocarpa	AMLE	Leaf
Gentiana lutea	GLE	Root
Helichrysum plicatum	HPE	Herba
Hypericum perforatum	HyPE	Herba
Aronia melanocarpa	AMFE	Fruit
Satureja subspicata	SSE	Herba
Mahonia aquifolia	MAE	Peel
Punica granatum	PPE	Peel
Vitis vinifera, var. Prokupac	WPE	Wine

2.2. Medicinal plants extract (MPE)

After drying at room temperature plant material was milled in a laboratory mill. Extraction was done using maceration with 70% ethanol (EtOH). The extracts were then filtered and the solvent was evaporated under low pressure at temperature not higher than 50 °C. Wine of the grape variety Prokupac was also evaporated to dryness under vacuum. Dry extracts were kept in a vacuum desiccator until analysis. Medicinal plants and their parts used for testing are listed in Table 1.

2.3. Antiviral activity of MPE

MPE have been diluted with two solvents: 5% dimethyl sulfoxide (DMSO) and combination of 30% ethanol in 5% DMSO to obtain final concentrations of 0.4; 1.0 and 2.5 mg/mL. To test the antiviral activity of MPE the equal volumes of hNoV and extracts suspensions have been mixed and incubated 1h at 37 °C. Solvents used for MPE preparation (5% DMSO, mixture of 30% ethanol in 5% DMSO) have been tested to confirm that they themselves have no effect on the reduction of hNoV particles.

2.4. Disinfectant solution and experimental treatment with hNoV suspension

Commercial household bleach was purchased at a local store (Belgrade, Serbia). The main ingredient of the bleach was sodium hypochlorite (NaOCl, 4.5% [w/v]). Experimental sodium hypochlorite solution was diluted with dimethyl sulfoxide (5% DMSO) to 250 ppm and 25 ppm free chlorine concentration. Equal volumes of hNoV suspension and disinfectant solution have been mixed and incubated 1h at 37 °C. Because of the possible aggression of free chlorine on isolation of hNoV RNA with commercial kit, the direct method with TRIzol^{TM} reagent has been used (Radin and D'Souza, 2011).

2.5. RT-qPCR assays

One-Step RT-qPCR assays were performed using 20 µL reaction mixture containing 10 µL of oasig TM qRT-PCR MasterMix, 1 µL of Norovirus Primer/Probe mix and hNoV RNA sample. No-template control (NTC) was added to verify the absence of contamination. Cycling conditions for RT-qPCR were as follows: reverse transcription at 55 °C/10 min, enzyme activation 95 °C/2 min, denaturation at 94 °C/12 min, followed by 50 cycles. RT-qPCR assays were done using Quantification of Norovirus genotype I and II (PrimerTMLtd, genesing, United Kingdom).

3. RESULTS AND DISCUSSION

3.1. Reduction of hNoV in suspension by medicinal plant extracts

Antiviral activity of 10 medicinal plant extracts applied at different concentrations (0.4, 1.0, 2.5 mg/mL) and dissolved in two different solvents has been examined for the reduction of foodborne hNoV particles in suspension following treatment at 37 °C during 1h (Table 2). The reduction of norovirus >1 $\log_{10}$ GE has been obtained with 1 mg/mL of *Aronia melanocarpa* leaf extract (AMLE), dried wine Prokupac (WPE) and *Hypericum perforatum* (HyPE) extract, and therefore could be promising for hNoV reduction having in mind that tested concentrations have been lower than usually used. The reduction in the interval 0.5 - 1 $\log_{10}$ GE has been obtained with 0.4 mg/mL of WPE, PPE and HyPE; 1.0 mg/mL of GAE and AMFE; 2.5 mg/mL of GAE and AMFE. All results of anti-norovirus activity of tested MPE which achieve < 0.5 $\log_{10}$ GE are considered as not effective. Some of MPE i.e. HyPE, *Aronia melanocarpa* fruit extract (AMFE) and *Punica granatum* (PPE) showed better activity when dissolved in 30% ethanol with 5% DMSO.

According to the literature, this is the first report of the anti-norovirus activity of *Aronia melanocarpa*. Antiviral activity of *A. melanocarpa* on some other viruses was reported. Park et al. (2013) reported that fruits of *A. melanocarpa* possess *in vitro* and *in vivo* activity against different subtypes of influenza viruses and such activity was also noticed for oseltamivir-resistant strain. They concluded that the activity was attributed to two constituents i.e. ellagic acid and myricetin.

Moreover, this is the first report of the anti-norovirus activity of the wine obtained from Serbian autochthonous grape variety Prokupac. It is in accordance with the Oh et al. (2015) findings that red wine as well as its component resveratrol can affect the foodborne viral surrogates, murine norovirus-1 and feline calicivirus Fs at an early stage of infection.

As for *Hypericum* species, Akram et al. (2018) assembled the facts from articles published in English language since 1982 to 2017 about antiviral potential of plants in various viral diseases such as influenza, hepatitis, human immunodeficiency virus (HIV), herpes simplex virus (HSV), etc. which have been proven in clinical studies. One of the species which was pointed out for its activity was *Hypericum connatum*. Also, antiviral activity was reported for other *Hypericum* species (de Carvalho Meirelles et al., 2019).

3.2. Virucidal effects of disinfectant solution against hNoV particles

As one kind of control, proven effective disinfectant against hNoV has been tested for the reduction of virus particles. Commercial household bleach containing sodium hypochlorite (NaOCl, 4.5% [w/v]) was diluted with dimethyl sulfoxide (5% DMSO) to 250 ppm and 25 ppm free chlorine concentrations. Equal volumes of hNoV suspension and disinfectant solution have been mixed and incubated 1h at 37 °C. At lower concentration disinfectant solution have reduced hNoV particles at the level of 0.62±0.01 $\log_{10}$ GE similar as some of MPE. The higher concentration of disinfectant solution showed significant reduction of hNoV particles at level of 2.40±0.02 $\log_{10}$ GE.

Similar results have been published by Kingsley et al. (2014) concluding that free chlorine treatments at concentrations of 33 and 189 ppm reduced hNoV binding in the PGM-MB assay by 1.48 and 4.14 $\log_{10}$, while 1 h treatment with 350 ppm chlorine dioxide reduced hNoV by 2.8 $\log_{10}$. According to Codex Alimentarius (2012) a biocide is considered effective when the log reduction of the infectious viral titer is >3 $\log_{10}$.

Table 2. Antiviral activity of medicinal plants extracts against foodborne norovirus after treatment at 37 °C 1h

	Concentration of medicinal plants extracts				
	0.4 [mg/mL]		1 [mg/mL]		2.5 [mg/mL]
	5% DMSO[a]	5% DMSO +30% EtOH	5% DMSO	5% DMSO +30% EtOH	5% DMSO
Extract	Reduction of hNoV[b,c,d] [log_{10} genome equivalents]				
SRE	0.33±0.01	NR	0.28 ±0.03	-	-
GAE	0.49±0.04	-	0.59±0.01	-	0.79±0.07
AMLE	0.45±0.02	-	1.06±0.05	-	-
GLE	0.45±0.01	-	0.46±0.01	-	0.26±0.04
WPE	0.79±0.02	0.30±0.01	1.21±0.01	-	-
HPE	-	0.48±0.03	0.22±0.02	-	0.32±0.01
HyPE	ND	0.93±0.01	ND	1.38±0.01	-
AMFE	0.12±0.03	0.28±0.03	0.29±0.05	0.66±0.02	0.55±0.02
SSE	0.23±0.01	NR	NR	-	0.13±001
MAE	NR	-	0.21±0.01	-	NR
PPE	0.70±0.03	0.83±0.03	-	-	-

[a]HNoV in 5% DMSO served as a control and had been compared with treatments of MPE.
[b]NR – no reduction
[c]ND - not detected
[d]- - not done

CONCLUSION

In conclusion, the most active extracts were *Aronia melanocarpa* leaves extract, *Hypericum connatum* extract, and dried Prokupac wine. It was noticed that the type of solvent used for dissolution made a significant difference in anti-noroviral activity. Some of the medicinal plant extracts showed anti-noroviral activity similar to the effect of lower concentration of sodium hypochlorite that stands as one of the few efficient chemical disinfectants but with byproducts that could be health hazardous.

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REFERENCES

Akram, M., Tahir, I. M., Shah, S. M. A., Mahmood, Z., Altaf, A., Ahmad, K., Munir, N., Daniyal, M., Nasir, S. and Mehboob, H. (2018). Antiviral potential of medicinal plants against HIV, HSV, influenza, hepatitis, and coxsackievirus: A systematic review, *Phytotherapy Research* **32**(5): 811–822.

Braga, L., Shupp, J., Cummings, C., Jett, M., Takahashi, J., Carmo, L., Chartone-Souza, E. and Nascimento, A. (2005). Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production, *Journal of Ethnopharmacology* **96**(1-2): 335–339.

Codex Alimentarius (2012). CAC/GL 79-2012 guidelines on the application of general principles of food hygiene to the control of viruses in food, *Codex Committee on Food Hygiene* **13**.

de Carvalho Meirelles, G., Bridi, H., Rates, S. M. K. and von Poser, G. L. (2019). Southern Brazilian *Hypericum* Species, Promising Sources of Bioactive Metabolites, *Studies in Natural Products Chemistry*, Vol. 59, Elsevier, pp. 491–507.

D'Souza, D. H. (2014). Phytocompounds for the control of human enteric viruses, *Current Opinion in Virology* **4**: 44–49.

EFSA/ECDC (2016). The european union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015, *The EFSA Journal* **14**(12): 4634.

El-Senousy, W. M., El-Gamal, M. S., Mousa, A. A. E.-B., El-Hawary, S. E.-S., Kamel, M. M., Fathi, M. N. and El-Mahdy, E.-M. M. (2014). Effect of Chlorine on Noroviruses, Rotaviruses and Hepatitis E Virus in Drinking Water, *World Applied Sciences Journal* **32**(11): 2206–2212.

Jaykus, L., D'Souza, D. and Moe, C. (2013). Foodborne viruses, *in* M. Doyle and R. Buchanan (eds), *Food Microbiology Fundamentals and Frontiers*, ASM Press, Washington, DC, USA.

Kingsley, D. H., Vincent, E. M., Meade, G. K., Watson, C. L. and Fan, X. (2014). Inactivation of human norovirus using chemical sanitizers, *International Journal of Food Microbiology* **171**: 94–99.

Oh, M., Lee, J.-H., Bae, S. Y., Seok, J. H., Kim, S., Chung, Y. B., Han, K. R., Kim, K. H. and Chung, M. S. (2015). Protective effects of red wine and resveratrol for foodborne virus surrogates, *Food Control* **47**: 502–509.

Papapanagiotou, E. (2017). Foodborne Norovirus State of Affairs in the EU Rapid Alert System for Food and Feed, *Veterinary Sciences* **4**(4): 61.

Park, S., Kim, J. I., Lee, I., Lee, S., Hwang, M.-W., Bae, J.-Y., Heo, J., Kim, D., Han, S.-Z. and Park, M.-S. (2013). Aronia melanocarpa and its components demonstrate antiviral activity against influenza viruses, *Biochemical and Biophysical Research Communications* **440**(1): 14–19.

Radin, D. and D'Souza, D. H. (2011). Simple and Rapid Detection of Human Norovirus from Produce Using SYBR Green I-based Real-time RT-PCR, *Food and Environmental Virology* **3**(3-4): 121–129.

Rocha-Pereira, J., Van Dycke, J. and Neyts, J. (2016). Norovirus genetic diversity and evolution: implications for antiviral therapy, *Current Opinion in Virology* **20**: 92–98.