5th International Electronic Conference on Medicinal Chemistry
1-30 November 2019
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

Pharma-toxicological and phytochemical investigations on Harpagophytum procumbens DC. ex Meisn. water extract: potential application in colon inflammation

**Giustino Orlando[1]*

[1] Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Chieti 66100, Italy.

* Corresponding author: giustino.orlando@unich.it

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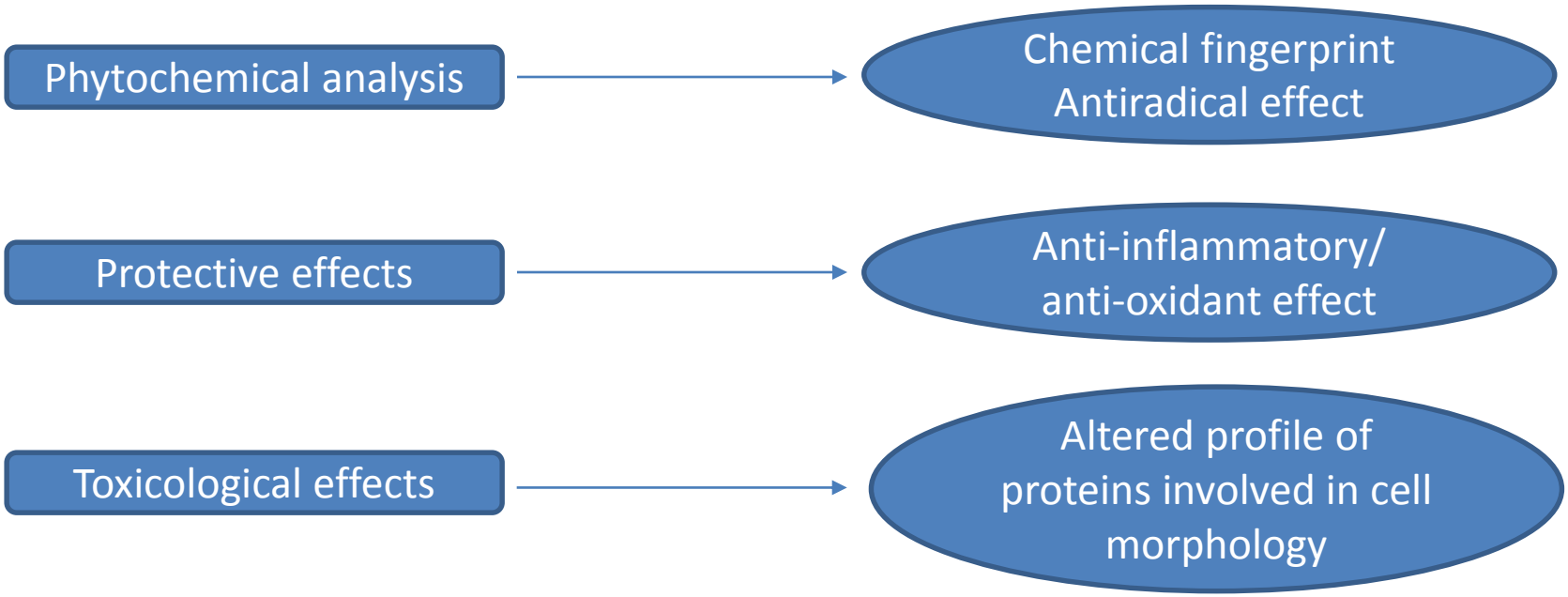
Graphical Abstract



Abstract

Inflammatory bowel diseases (IBDs) are chronic, relapsing and multifactorial disorders of the colonic mucosa, which show increased and unbalanced intestinal immune response to external stimuli. Plant-derived extracts were described to possess the capability in contrasting IBDs-related oxidative stress and inflammatory pathways. In the present study, we investigated the water extract of *Harpagophytum procumbens* DC. ex Meisn. in an experimental model of IBD. Additionally, a microbiological investigation was carried out to discriminate the efficacy against bacterial and fungal strains involved in IBDs. Finally, an untargeted proteomic analysis was conducted on more than 100 colon proteins involved in tissue morphology and metabolism. The extract showed the ability to blunt the level of selected biomarkers of oxidative stress and inflammation, including serotonin, prostaglandins, cytokines and transcription factors. Additionally, the extract inhibited the growth of *Candida albicans* and *C. tropicalis*, *in vitro*. The extract was also able to exert a pro-homeostatic effect on the levels of a wide plethora of colon proteins, thus corroborating protective effects against the burden of inflammation and oxidative stress. On the other hand, the supra-physiological downregulation of cytoskeletal-related proteins involved in tissue morphology and antimicrobial barrier function suggests caution in the use of food supplements enriched with *H. procumbens.*

Keywords: *Harpagophytum procumbens;* IBDs; Oxidative stress; Inflammation; Proteomic analysis.

Introduction

Inflammatory bowel diseases (IBDs) are chronic, relapsing and multifactorial disorders of the colonic mucosa (ulcerative colitis), which show increased and unbalanced intestinal immune response to external stimuli. As a consequence of this condition, colon mucosa produces numerous pro-inflammatory biomarkers, including reactive oxygen/nitrogen (ROS/RNS) species, prostaglandins and cytokines, which reinforce the inflammatory status, thus causing tissue damage. At the moment, the first choice drugs for treating IBDs are aminosalycilates, glucocorticoids, immune-suppressants and tumor necrosis factor (TNF)α inhibitors. Nevertheless, numerous patients (20-40%) experience the lack of efficacy or side effects, thus highlighting the urgent need of novel therapies, which could both implement the efficacy and reduce the incidence of side effects. Plant-derived extracts have long been described to possess the capability in contrasting IBDs-related oxidative stress and inflammatory pathways. To this regard, it is of noteworthy interest to treat inflammatory conditions through home- made extracts prepared from plants traditionally used by folk populations. These extracts, especially those prepared with traditional and biocompatible solvents (water, hydroalcoholic solutions) in the forms of infusions or decoctions, could not only join efficacy and safety, due to their consolidated use in the population, but also represent innovative approaches for improving and valorizing local botanical resources and productive chains.

In the present study, we further deepened the protective effects of the previously described water extract of *Harpagophytum procumbens* DC. ex Meisn., also known as devil's claw, in an *ex vivo* experimental model of colon inflammation constituted by isolated rat colon challenged with *E. coli* lipopolysaccharide (LPS). In particular, the water extract of *H. procumbens* was further assayed for the determination of plant secondary metabolites belonging to the classes of phenols and flavonoids, namely gallic acid, resveratrol, catechin and epicatechin. as well as the iridoid compound harpagoside. Harpagoside is considered the main responsible of the therapeutic activity of the plant, therefore the measurement of its content (not lower than 1.2% *w/w*) in the extract represents an evaluation of the qualitative standard described in European Pharmacopoeia (Menghini et al., 2019). In addition, we further investigated the possible mechanisms of the extract of *H. procumbens* on multiple inflammatory and oxidative stress pathways, by measuring production of colon serotonin (5-HT), prostaglandin (PG)E_2 and 8-iso-$PGF_{2\alpha}$, as well as tumor necrosis factor a (TNFa), nuclear factor kappa B (NFkB), interleukin (IL)-6 and nuclear factor erythroid 2–related factor 2 (Nrf2) mRNA levels. An untargeted proteomic analysis was also performed in order to explore the putative mechanism in the colon. To this regard, the proteomic investigation was carried out on a cluster of more than one hundred proteins involved in colon cell morphology and metabolism. Finally, the extract was subjected to a microbiological pilot study, as well, in order to evaluate the possible inhibitory role on specific bacterial strains and fungi involved in IBDs, including ulcerative colitis, such as *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, and *C. tropicalis*.

Results and discussion

The extract showed the ability to blunt the level of selected biomarkers of oxidative stress and inflammation, including serotonin, prostaglandins, cytokines and transcription factors. Additionally, the extract inhibited the growth of *Candida albicans* and *C. tropicalis*, *in vitro*. The extract was also able to exert a pro-homeostatic effect on the levels of a wide plethora of colon proteins, thus corroborating protective effects against the burden of inflammation and oxidative stress. On the other hand, the tested extract was ineffective against a limited number (N=4) of proteins, whereas it determined a supra-physiological alteration, of 30 proteins whose levels were not altered by the LPS stimulus. Particularly, our attention focused on a cluster of proteins namely ezrin (EZRI), actin-related protein 2/3 complex subunit 4 (ARPC4), plastin-1 (PLSI), smoothelin (SMTN) which are involved in tissue morphology through multiple regulatory functions on cytoskeletal formation. The alteration of the physiological protein level exerted by extract treatment could be at the basis of potential morphological alterations of colon tissue. Additionally, according to the putative role of alpha-defensin 8 (DEFA8) and alpha-defensin 11 (DEFA11) in the colon (data reported in the recognized database “uniprot.org”), the supra-physiological downregulation of their levels after extract challenging could negatively influence the function of the intestinal antimicrobial barrier. Therefore, the downregulation of DEFA8 and DEFA11 colon levels induced by the extract could paradoxically lead to the onset of favourable conditions for the development of opportunistic pathogens, thus contrasting the observed intrinsic antimicrobial effects.

Phytochemical analysis

	Total phenols		Total flavonoids	
	mg/g extract	S.D.	mg/g extract	S.D.
H. procumbens	65.7	6.9	5.4	1.9

Phytochemical analysis

Phenolic content of H. procumbens extract		
	mg/g extract	S.D
Gallic acid	9.74	0.88
Catechin	2.90	0.35
Epicatechin	3.03	0.18
Resveratrol	3.33	0.33

Phytochemical analysis

	DPPH		ß-carotene/linoleic acid	
	IC_{50} µg/ml	S.D.	IC_{50} µg/ml	S.D.
BHT			2.5	0.32
Trolox	4.07	0.44	4.03	0.57
H. procumbens	121.01	16.6	16.8	2.05

Microbiological studies

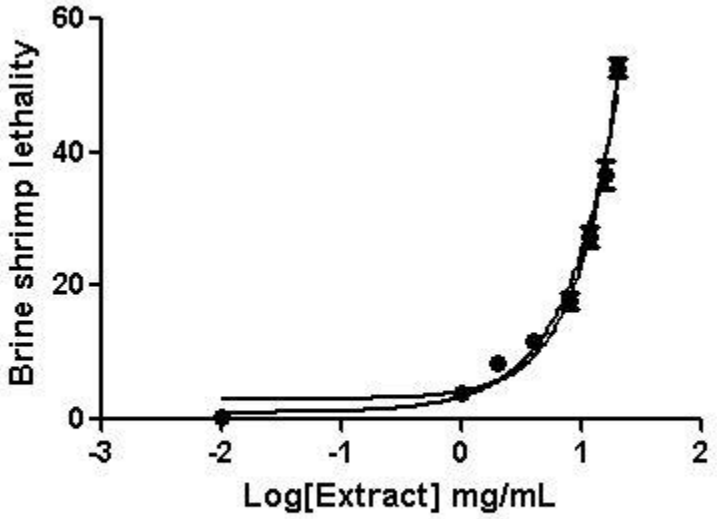
	MIC[1] (μg/ml)*		
	H. procumbens	Ciprofloxacin	
E. coli (clinical isolate)	11.80 (9.37-18.75)	<0.12	
P. aeruginosa (clinical isolate)	188.98 (150-300)	<0.12	
S. aureus (ATCC 6538)	> 300	0.98	
* MIC values are reported as geometric means of three independent replicates (n=3); MIC range concentrations are reported within brachets.			

Microbiological studies

	MIC[1] (µg/ml)*	
	H. procumbens	Fluconazole
C. albicans (YEPGA 6183)	11,80 (9.37-18.75)	2
C. tropicalis (YEPGA 6184)	5.89 (4.68-9,37)	4

* MIC values are reported as geometric means of three independent replicates (n=3); MIC range concentrations are reported within brachets.

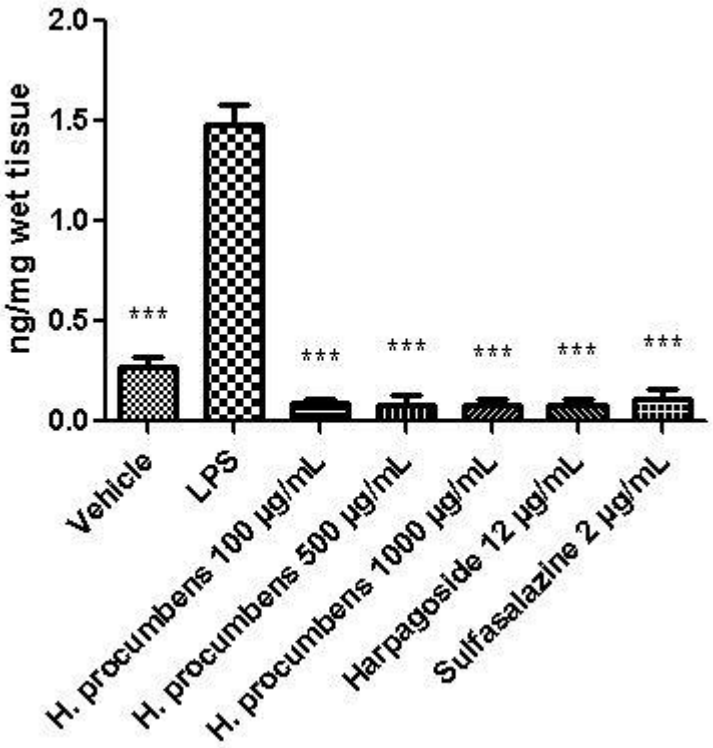
In vitro studies



H. procumbens (16 mg/mL < LC_{50} < 20 mg/mL)

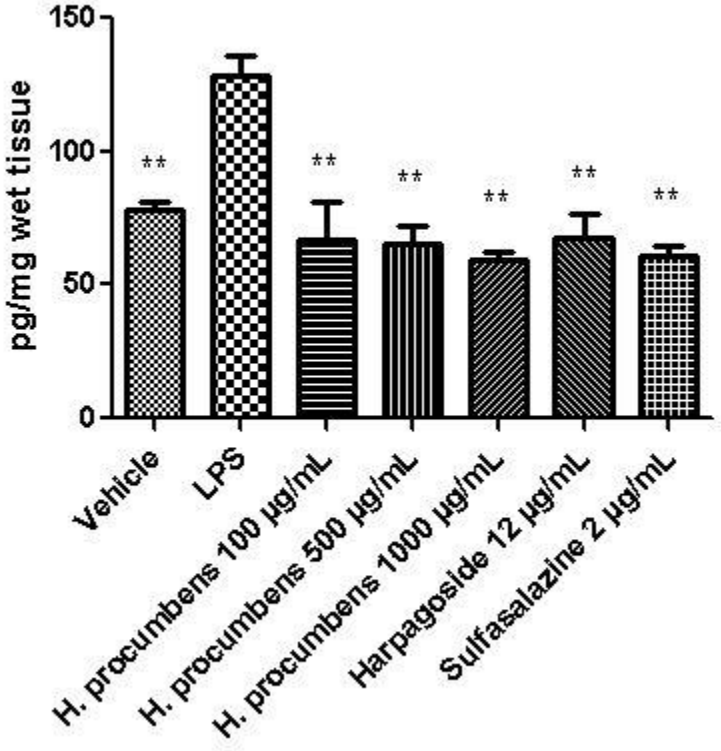
Effects of water *H. procumbens* extracts (0.1-20 mg/mL) on Artemia salina Leach viability (Brine shrimp lethality test). Data are means ± SD of three experiments performed in triplicate.

Ex vivo studies: IBD model



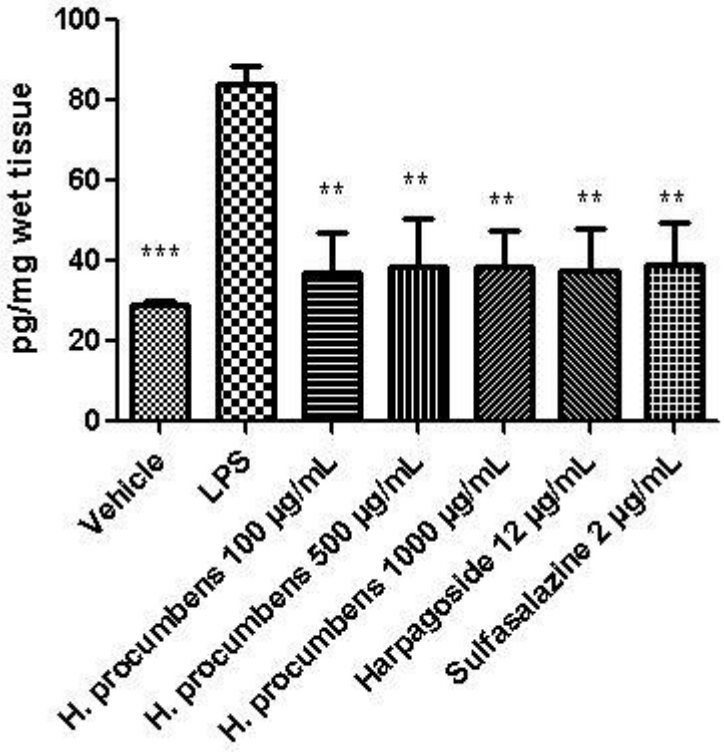
Effect of water *H. procumbens* extract (100-1000 µg/mL) on serotonin (5-HT) level (ng/mg wet tissue) in mouse colon specimens challenged with LPS. ANOVA, P<0.0001; post-hoc, ***P<0.001 vs. LPS.

Ex vivo studies: IBD model



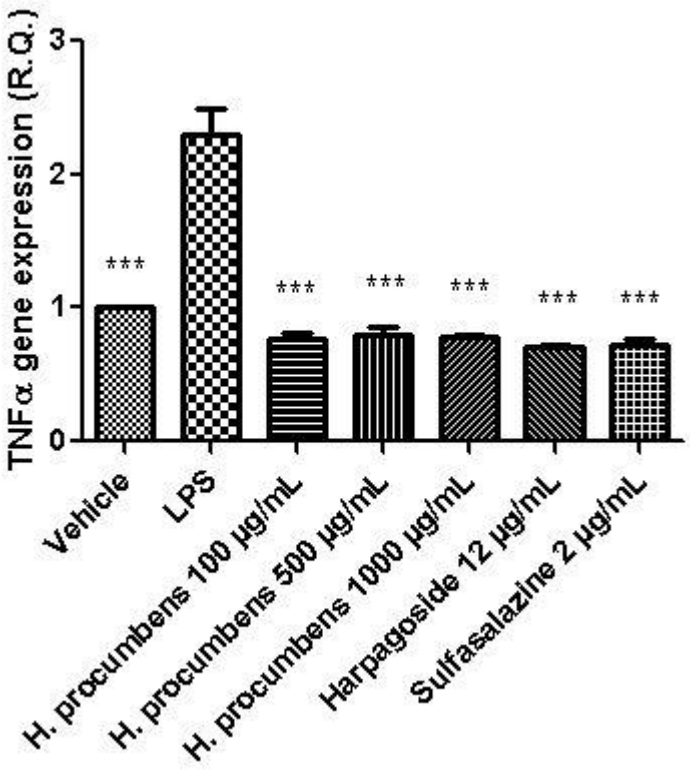
Effect of water *H. procumbens* extract (100-1000 µg/mL) on prostaglandin (PG)E_2 level (pg/mg wet tissue) in mouse colon specimens challenged with LPS. ANOVA, P<0.001; post-hoc, **P<0.01 vs. LPS.

Ex vivo studies: IBD model



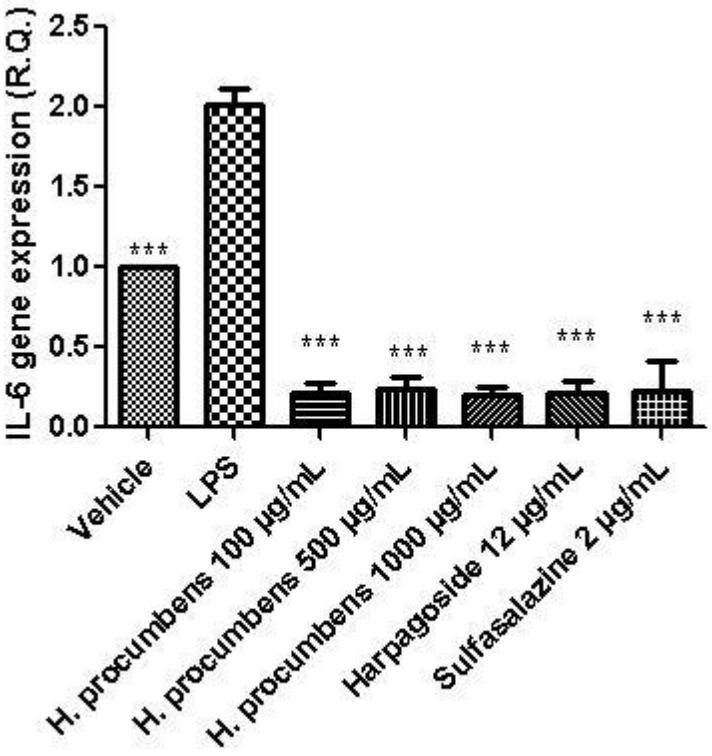
Effect of water *H. procumbens* extract (100-1000 µg/mL) on 8-iso-prostaglandin (PG)$F_{2\alpha}$ level (pg/mg wet tissue) in mouse colon specimens challenged with LPS. ANOVA, P<0.001; post-hoc, **P<0.01, ***P<0.001 vs. LPS.

Ex vivo studies: IBD model



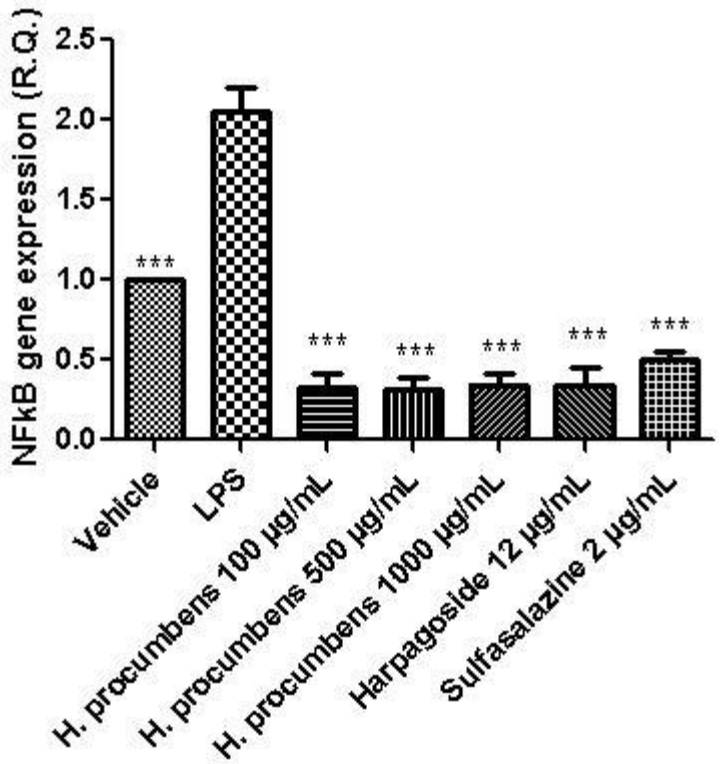
Effect of water *H. procumbens* extract (100-1000 µg/mL) on tumor necrosis factor (TNF)α gene expression (relative quantification) in mouse colon specimens challenged with LPS. ANOVA, P<0.001; post-hoc, ***P<0.001 vs. LPS.

Ex vivo studies: IBD model



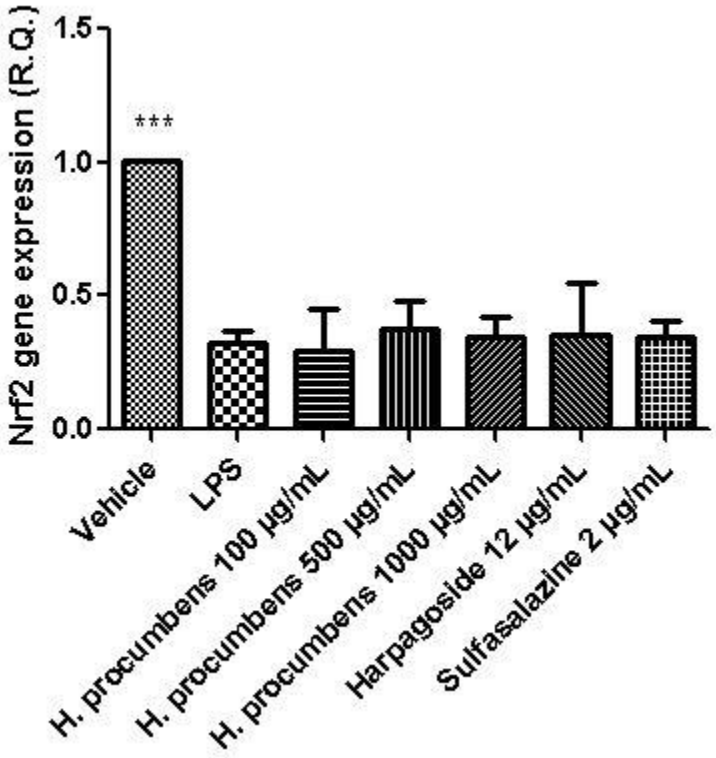
Effect of water H. procumbens extract (100-1000 µg/mL) on interleukin (IL)-6 gene expression (relative quantification) in mouse colon specimens challenged with LPS. ANOVA, P<0.001; post-hoc, ***P<0.001 vs. LPS.

Ex vivo studies: IBD model



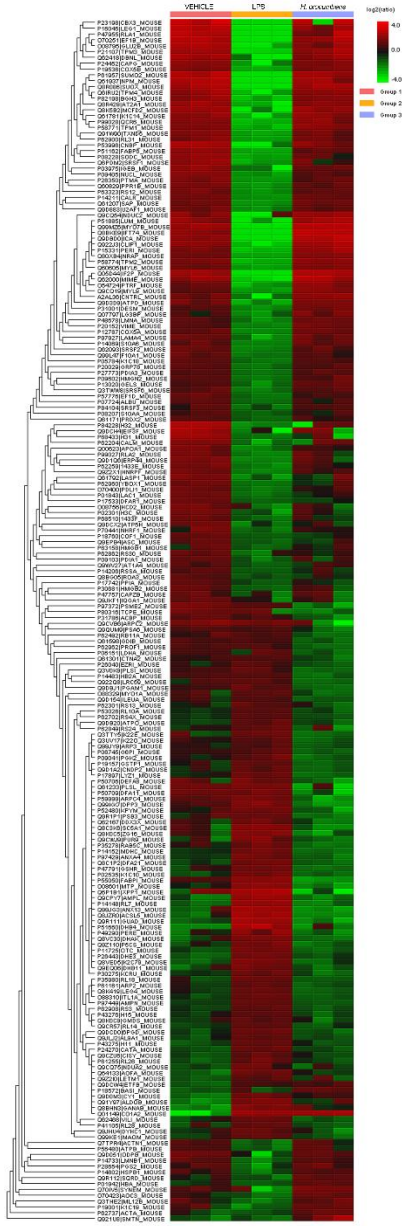
Effect of water H. procumbens extract (100-1000 µg/mL) on nuclear factor kappa B (NFkB) gene expression (relative quantification) in mouse colon specimens challenged with LPS. ANOVA, P<0.001; post-hoc, ***P<0.001 vs. LPS.

Ex vivo studies: IBD model



Effect of water H. procumbens extract (100-1000 µg/mL) on nuclear factor erythroid 2–related factor 2 (Nrf2) gene expression (relative quantification) in mouse colon specimens challenged with LPS. ANOVA, P<0.001; post-hoc, ***P<0.001 vs. LPS.

Ex vivo studies: IBD model



The levels of the identified proteins were expressed relatively to the LPS group (positive control) and depicted in the figure. The green bar indicates a down-regulating effect compared to LPS, whereas the red one indicates an up-regulating effect. It was observed that extract treatment showed the ability to exert a pro-homeostatic effect on most of the quantified proteins. Particularly, the extract blunted the alteration of protein level induced by LPS, thus restoring the physiological condition observed in the vehicle-treated group. It is of noteworthy interest the pro-homeostatic effect on the levels of peroxiredoxin-2 (PRDX2), glutathione reductase (GSHR), catalase (CATA) and superoxide dismutase (SODC), which are deeply involved in contrasting oxidative stress-induced organ injury. In addition, the water extract of *H. procumbens* normalized the levels of specific proteins namely drebrin-like protein (DBNL), macrophage-capping protein (CAPG), prothymosin alpha (PTMA) and high mobility group protein B2 (HGMB2) which are involved in the anti-proliferative effect, T-cell regulation and defence against opportunistic infections. Collectively, the pro-homeostatic effect exerted by the extract on the selected proteins is consistent with the reported anti-radical/anti-oxidant, anti-inflammatory and anti-micotic effects. Moreover, considering the involvement of CAPG in the colon cancer progression, the present proteomic analysis further corroborates the anti-proliferative effect exerted by *H. procumbens* water extract (1 mg/mL) on human colon cancer HCT116 cell line. On the other hand, the tested extract was ineffective against a limited number (N=4) of proteins, whereas it determined a supra-physiological alteration of about 30 proteins, whose levels were not modified by the LPS stimulus. Particularly, our attention focused on a cluster of proteins namely ezrin (EZRI), actin-related protein 2/3 complex subunit 4 (ARPC4), plastin-1 (PLSI), smoothelin (SMTN) which are involved in tissue morphology through multiple regulatory functions on cytoskeletal formation. The supra-physiological alteration of protein level exerted by extract treatment could be at the basis of potential morphological alterations of colon tissue. Additionally, the extract was also able to reduce the physiological concentration of colon alpha-defensin 8 (DEFA8) and alpha-defensin 11 (DEFA11). According to the putative role exerted by DEFA8 and DEFA11 in improving the function of the intestinal antimicrobial barrier (data reported in the recognized database "uniprot.org"), the supra-physiological downregulation of their levels after extract challenging could paradoxically lead to the onset of favourable conditions for the development of opportunistic pathogens, thus contrasting the observed intrinsic antimicrobial effects.

Conclusions

Concluding, the present multidirectional study showed protective effects of *H. procumbens* water extract in blunting the burden of oxidative stress and inflammation in LPS-stimulated colon, alongside with antimicrobial effects against pathogen fungal strains involved in IBD. Additionally, the fingerprint phytochemical analyses suggest the involvement of multiple active principles namely harpagoside, gallic acid, catechin, epicatechin and resveratrol in the observed pharmacological effects.

Nevertheless, the supra-physiological downregulation of EZRI, ARPC4, PLSI, SMTN, DEFA8, DEFA11 after extract treatment indicates potential morphological alterations in the colon tissue that should be taken in account in further researches. In this context, the present study recommends caution in the use of food supplements enriched with *H. procumbens.*

Acknowledgments

This work was supported by grants from the Italian Ministry of University (FAR 2019 granted to Claudio Ferrante; FAR 2019 granted to Annalisa Chiavaroli; FAR 2019 granted to Luigi Menghini; FAR 2019 granted to Giustino Orlando; FAR 2014 granted to Lucia Recinella).