Article published on 2024-6-21

Formulation and Evaluation of Drumstick Leaves Tablet as An Immunomodulator

V.R. Ravikkumar[1], Bhoomi D. Patel[2*], Sanjesh Rathi[7], S. Parthiban[3], Mohini C. Upadhye[4], Apexaben M. Shah[5], Shaheen Shaik Abdul Rehan[6], Sayan Samanta[8], Shubham Singh[7]

[1]Department of Pharmacognosy, The Erode College of Pharmacy,Erode, Tamilnadu, India-638112.
[2*]Department of Pharmaceutical Chemistry & Quality Assurance, School of Pharmacy, Rai University, Saroda, Dholka Road, Ahmedabad, Gujarat, India-382260.
[3]Department of Pharmaceutics, PGP College of Pharmaceutical Science and Research Institute, Namakkal, Tamilnadu, India-637207.
[4]Department of Pharmacognosy, PES Modern College of Pharmacy (For Ladies), Moshi, Pune, India.
[5]Department of Pharmaceutics, Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India-382355.
[6]Shadan Women's College of Pharmacy, Telangana, Hyderabad, India - 500004.
[7]Department of Pharmaceutics, School of Pharmacy, Rai University, Saroda, Dholka Road, Ahmedabad, Gujarat, India-382260.
[8]Department of Pharmacy, School of Pharmacy, Rai University, Saroda, Dholka Road, Ahmedabad, Gujarat, India-382260.

***Correspondence Author:**
Dr. Bhoomi Dineshkumar Patel
Email: bhoomipatel2512@gmail.com
Department of Pharmaceutical Chemistry & Quality Assurance, School of Pharmacy, Rai University, Saroda, Dholka Road, Ahmedabad, Gujarat, INDIA-382260.

Chinese Journal of Applied Physiology, 2024: e20240004

Abstract The human immune system plays a pivotal role in protecting the body against pathogens, maintaining homeostasis, and preventing disease. Immunomodulation, the process of regulating immune responses, is crucial for optimal health. In recent years, there has been growing interest in natural remedies for immune system modulation, driven by the recognition of their potential efficacy and safety profiles.

This project aims to investigate the immunomodulatory effects of drumstick leaves tablets, derived from Moringa oleifera, a plant known for its rich nutritional and medicinal properties. The study will explore the potential of drumstick leaves tablets to modulate immune responses through in vitro and in vivo experiments.

Through comprehensive analysis of the immunomodulatory properties of drumstick leaves tablets, this project aims to contribute to our understanding of natural remedies for immune system modulation. The findings could have significant implications for the development of novel therapeutic interventions aimed at enhancing immune function and improving human health.

Keywords Drumstick, Immunomodulator, *Moringa oleifera*, Formulation, Evaluation, Tablet

DOI: 10.62958/j.cjap.2024.004
www.cjap.ac.cn

Published by CJAP editorial office and Asian BioMed Innovation Press

1. INTRODUCTION

1.1 *Moringa oleifera*

Moringa oleifera Lam. is a fast-growing, drought-resistant tree of the family Moringaceae, native to the Indian subcontinent and used extensively in South and Southeast Asia. Common names include moringa, drumstick tree, horseradish tree, or malunggay [1]. It is widely used in the chemical, food, and fuel industries [2]. Its main characteristics are its high nutritional value and medicinal, functional, and coagulant properties.[3] Each part of the *M. oleifera* plant (Example: roots, stems, leaves, flowers, green pods and seeds) has one or more uses with different properties.

M. oleifera leaves and buds were used against headache by rubbing them on the temples. Roots and root barks were used as anti-scorbutic [4]. The eye diseases were treated with the juice of the leaves added with honey. Dried seeds of *M. oleifer*a were used in ophthalmic preparation, venereal affection anti-inflammatory and purgative and as tonic [5].

Leaves are feathery, pale green, compound, tripinnate, (30–60 cm long), with many small leaflets, 1.3 -- 2 cm long, 0.6 -- 0.3 cm wide, lateral ones slightly elliptic, terminal ones obovate, and slightly larger. The feathery leaves of the tripinnate complex have green curved leaflets that are 1–4 cm long. Because of its leaves, the tree is frequently mistaken for a leguminous plant. The alternate twice or thrice pinnate leaves appear at the branch tips in most cases. They have a long petiole with 8–10 pairs of pinnae, each bearing two sets of inverse elliptic leaflets and one at the apex and are 20–70 cm long when young [6].

T.R. Prashith Kekuda et al. (2010) investigated antibacterial and antifungal efficacy of steam distillate of Moringa oleifera. Among bacteria tested, more inhibition was observed in case of E. coli followed by S. aureus, K. pneumoniae, P. aeruginosa and B. subtilis. Inhibition of fungi was observed as reduced colony diameter in plates poisoned with distillate as compared to control plates. More inhibition of A. niger was observed followed by A. oryzae, A. terreus and A. nidulans [7-9].

M. F. Alam et al. (2018) investigated antibacterial activity of leaf juice and extracts of Moringa oleifera using disc diffusion and minimum inhibitory concentration (MIC) determination method against human pathogenic bacteria [10-12].

S.G. Mahajan et al. (2017) reported anti-arthritic activity of ethanolic extract of seeds of Moringa oleifera Lam. in adjuvant-induced arthritis in adult female Wistar rats [13-15].

S.G. Mahajan et al. (2019) evaluated anti-inflammatory activity from the n-butanol extract of the seeds of Moringa oleifera against ovalbumin-induced airway inflammation in guinea pigs [16].

M. Bajpai et al. (2015) reported antioxidant activity from the leaves of Moringa oleifera. The antioxidant property was found due to the presence of kaempferol [17].

T. Rastogi et al. (2019) reported anthelmintic activity of Moringa oleifera. Ethanolic extracts of Moringa oleifera were taken for anthelmintic activity against Indian earthworm Pheritima posthuma. Various concentrations of extract were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as a reference standard and distilled water as a control group [18-20].

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

For the study the leaves of Moringa oleifera were collected from the matured tree from the campus of Rai University with the permissions of officials within the plantation of Garden of Rai University, Ahmedabad.

2.2 Preparation of Sample

After collection, the leaves were removed from the branches, sorted out. Washed properly with sterile water and for drying spread on a mesh tray. The leaves were shade dried for a period of 7- 10 days for proper drying in the laboratory. Upon drying, the leaves were pulverized under aseptic conditions using a grinder, the fine powder sieve and stored in a dry airtight glass jar for phytochemical and nutritional analyses.

2.3 Analysis of Sample

2.3.1 Test for Carbohydrate

MOLISCH TEST: Take 5 ml of sample solution in a test tube and add a few drops of Molisch reagent. Mix and wait for a few minutes. If there is formation of a purple ring at the interface, then carbohydrate is present.

2.3.2 Test for Reducing And Non Reducing Sugar

BARFOED'S TEST: Take a small amount of sample solution (about 1 mL) in a test tube and add a few drops of Barfoed's reagent. Heat the mixture gently. Observe if there is formation of a brick-red precipitate. The formation of precipitate shows the presence of reducing/non reducing sugar.

2.3.3 Test for Proteins

BIURET TEST: Take about 2 mL of the sample solution in a test tube and add a few drops of Biuret reagent. Mix gently and observe for a color change to purple, indicating the presence of proteins or peptides.

2.3.4 Test For Glycoside

HCL TEST: Take the sample and dissolve it in a solvent like water or alcohol, (herr alcohol) and add a few drops of a hydrochloric acid (HCl) or sulfuric acid (H2SO4) for general detection. Heat the mixture gently. Observe any color changes or precipitate formation. Positive results, indicating the presence of glycosides, may include color changes ranging from yellow to brown or the formation of a precipitate.

2.3.5 Test for Phenol

Ninhydrin Test: Dissolve the sample in a suitable solvent, such as ethanol and add a few drops of ninhydrin solution to the sample. Heat the mixture gently. Observe the color change in the solution. If amino acids or proteins are present, a purple or blue color will develop. This color change indicates the presence of primary amines, which react with ninhydrin to form a colored complex.

Ferric Chloride: Take about 5 mL of the sample solution in a test tube and add a few drops of ferric chloride solution. A color change to violet, blue, green, or red indicates the presence of phenolic compounds.

2.4 Compounds Required for the Formulation

Tablet formulation requires several key components to ensure the tablet's efficacy, stability, and proper delivery of the active ingredients. Firstly, the active pharmaceutical ingredient (API) here is Drum stick leaves powder (our sample), providing the desired pharmacological effect. Excipients such as binders, fillers, and lubricants & glidants used here are Sodium Benzoate, Talc, Magnesium Stearate, Starch, Mannitol.

Table 1. Components forFormulation of tablet

Components	Quantity	
	1 Tablet	20 Tablets
Drum stick leaves powder	30 mg	600 mg
Sodium Benzoate	50 mg	1000 mg
Talc	10 mg	200 mg
Magnesium Stearate	10 mg	200 mg
Starch	60 mg	1200 mg
Mannitol	340 mg	6800 mg

2.5 Method for Preparation of Tablets

The weight mentioned in the steps are for 20 tablets each of 500mg.

1. Raw Material Selection: Choosing good quality herbal powders that we prepared, finely sieved. Here we took 600 mg of Drumstick leaves powder.

2. Mixing: Blend the herbal powders with any excipients or binders needed for the tablet formulation. This step ensures uniform distribution of ingredients. Here we are taking Sodium benzoate 1000mg, magnesium stearate 200mg, starch for slugging weighing 1200 mg. Adding mannitol as filler as well as flavoring agent weighing 6800 mg.

3. Granulation: Pass the blended powders through a roller compactor or a tablet press to form granules. This process breaks down large particles and enhances flow properties.

4. Sizing: Screen the granules to obtain particles of uniform size. This step helps in achieving consistent tablet weight and content uniformity.

5. Blending: Blend the sized granules with lubricants to improve tablet compression and dissolution properties. Here we used talc as lubricant weighing 200 mg.

6. Compression: Compress the granules into tablets using a tablet press machine.

7. Quality Control: Perform quality tests such as weight variation, hardness, disintegration, and dissolution to ensure the tablets meet the required specifications.

3. RESULTS AND DISCUSSION

3.1 Analysis of Sample

Analysis of sample by Preliminary testing and IR Spectroscopy which shown in table 2 and figure 1. IR spectroscopy performed by Press plate technique.

3.2 Evaluation Parameters

3.2.1. Bulk Density

Weight 10 gm of prepared formulation powder, and transfer it to the measuring cylinder.

Bulk Density= Weight taken/ Bulk

$= 10gm/ 4.5cm^3$

$=2.22\ gm/cm^3$

3.2.2 Tap Density

Weight 10 gm of prepared formulation powder, and transfer it to a measuring cylinder and put it in a density apparatus for 100 taps.

Tap Density= Weight taken/ Tap

$=10gm/ 3.5cm^3$

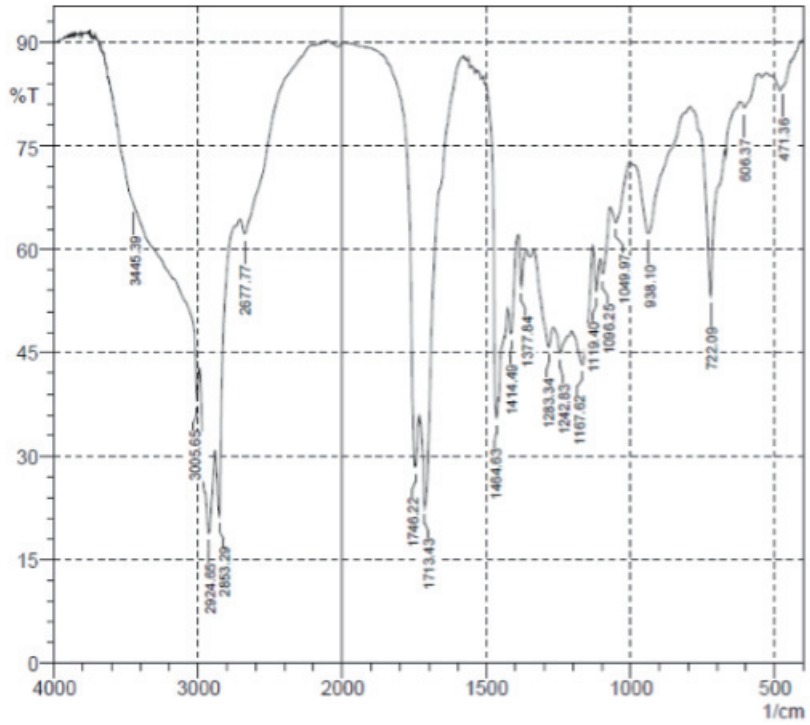

Figure 1. Observed FTIR-spectrum of leaf part of Moringa oleifera extract.

Table 2. Preliminary Identification test of Morienga Oliefera

Drug Powder	Reagents	Observation	Indications
Morienga Oliefera powder	Molish Test (Carbohydrates)	Purplr layer colour produce	(+)
Morienga Oliefera powder	Barfoed's Test (Reducing and non-reducing sugar)	Gives ppt	(+)
Morienga Oliefera powder	Iodine Test (Polysachharide)	Colour change to light brown	(+)
Morienga Oliefera powder	Biuret Test (Glycoside)	Purple Colour	(+)
Morienga Oliefera powder	Ninhydrin Test (Amino Acid)	Dark brown colour	(+)
Morienga Oliefera powder	Ferric Chloride (Phenols)	Dark black	(+)

Table 3. Angle of Repose

Angle of Repose	Observed	Flow Property
35-40	38.65	Fair Aid Not Needed

$=2.85\ gm/cm^3$

3.2.3 Angle of Repose

Angle of repose = tan-1 height/radius

θ= tan-1 4.5 /5.5

θ= 38.65°

3.2.4 Carr's Index

Carr's index = Tap density - Bulk density / Tap density x 100

= 2.85 - 2.22 / 2.85 x 100

= 22.1%

3.2.5 Hausner's Ratio

Hausner's ratio= Tap density / Bulk density

= 2.85 / 2.22

= 1.28

3.2.6 Friability

Accurately the tablet samples were weighed (20 tablets), and placed in the drum. The drum was rotated for 100 times, and then removed the tablets.

Friability % = Initial weight - Final weight / Initial weight x 100

= 9840 mg - 9770 mg / 9840 mg x 100

= 70/9840 x100

= 0.71%

3.2.7 Disintegration Test

Instrument Observation:

3.2.8 Disolution Test

Table 4. Carr's Index

Carr's Index	Observed	Flow Property
21-25	22.1	Passable

Table 5. Hausner's Ratio

Hausner's Ratio	Observed	Flow Property
1.26-1.34	1.28	Passable

Table 6. Friability

Acceptance Limit	Observed	Friability
N.m.t. 1% (Ip)	0.71%	Accepted

Table 7. Disintegration Test

Type Of Tablet	Disintegration Media	Disintegration Time (Ip)
Uncoated	1.2 Ph	30 Mins Or Less

Figure 2. Time duration observed: 28:10.91 mins.

Table 8. Disolution Test

Dissolution medium	As specified in the individual monograph
Medium dissolution of volume	900 ml
Total number of tablets	3
Time	1 hr for conventional release, minium 6 hrs for sustained release
Sampling interval	5 to 10 min.s for conventional release, minium 1 hr for sustained release
Temperature	36.5 -37.5 c

Table 9. Disolution Test: Time vs %CDR

Sr. No.	Time (min)	%CDR
1.	5	69.60221
2.	10	78.49718
3.	15	84.45599
4.	20	86.35709
5.	30	87.2717
6.	40	89.29926
7.	60	95.9984

Table 10. Apparatus used in Dissolution test

Usp appratus	Description	Rotating speed	Dosage form
Type I	Paddle apparatus	25 – 50 rpm	Orally disintegrating tablet

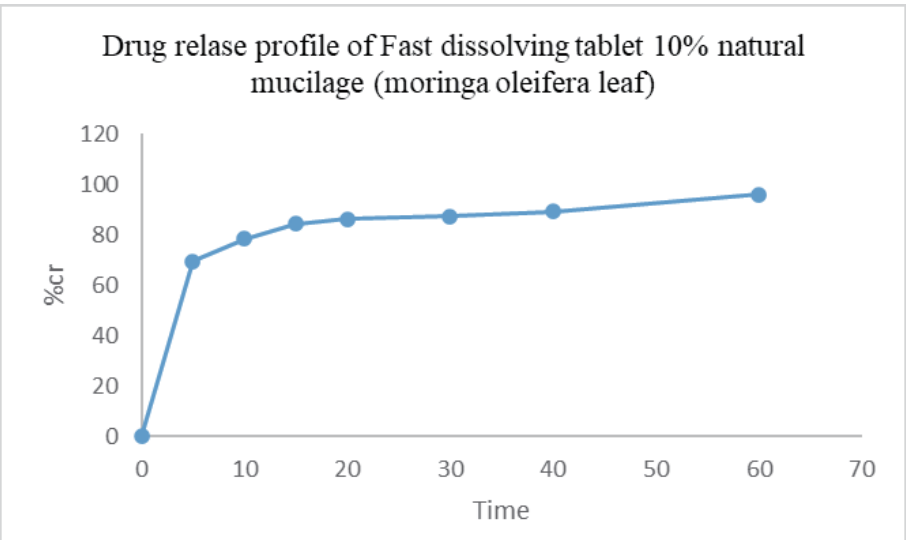

Figure 3. Time vs % CR graph

3. SUMMARY

Table 11. Summary of evaluation parameters

Sr. No.	Evaluation parameters	Result
1.	Bulk density	2.22 gm/cm^3
2.	Tap density	2.85 gm/cm^3
3.	Angle of repose	38.65° (fair aid not needed)
4.	Carr's index	22.1% (passable)
5.	Hausner's ratio	1.28 (passable)
6.	Friability	0.71% (accepted)
7.	Weight variation	1.01% (accepted)
8.	Disintegration test	30 mins or less (time)
9.	Disolution test	9984.9984 (%cdr)

4. CONCLUSION

In conclusion, our project has focused on formulating a herbal tablet using drumstick leaves powder as a potent immunomodulator. This initiative stems from the growing interest in natural remedies and traditional medicine for promoting immune health. Through a methodical approach encompassing extraction, formulation, and evaluation, we have successfully developed a herbal tablet that harnesses the immunomodulatory properties inherent in drumstick leaves.

Our findings highlight the effectiveness of the herbal tablet in modulating the immune system. The inclusion of drumstick leaves powder has demonstrated remarkable potential in enhancing immune function, thereby offering a natural and holistic approach to immune support.

Moreover, the formulation of this herbal tablet capitalizes on the rich phytochemical profile of drumstick leaves, including vitamins, minerals, and bioactive compounds known for their immunomodulatory effects. This natural synergy presents a compelling alternative to conventional immunomodulatory agents, with the added advantage of being safe, affordable, and easily accessible.

While our study marks a significant stride in exploring the immunomodulatory properties of drumstick leaves, there remain avenues for further research. Future investigations could delve into optimizing the formulation for enhanced bioavailability and efficacy, as well as conducting clinical trials to validate its therapeutic potential in humans.

In essence, the formulation of a herbal tablet using drumstick leaves powder as an immunomodulator holds promise as a sustainable and culturally relevant approach to immune health. By integrating traditional knowledge with contemporary scientific methodologies, we aspire to contribute to the development of natural remedies that prioritize wellness and resilience in the face of health challenges.

Competing Interest Statement

The authors declare no conflict of interest.

Acknowledgment

The Authors would like to thanks Dept. of Pharmachemistry and Quality Assurance, School of Pharmacy, Rai University, Ahmedabad for continuous assistance and support.

References

1. Olson, M.E.; Fahey, J.W. Moringa oleifera: Un árbol multiusos para las zonas tropicales secas. Rev. Mex. Biodivers 2011, 82, 1071–1082.
2. Granella SJ, Bechlin TR, Christ D, Coelho SR, de Oliveira Paz CH. An approach to recent applications of Moringa oleifera in the agricultural and biofuel industries. South African Journal of Botany. 2021 Mar 1;137:110-6.
3. Yadava, U.L. Exotic horticultural plants with commercial potential in the UnitedStates market: Introduction to the Workshop. HortScience 1996, 31, 764–765.
4. Alli, E.; And, R.; Arumugam, T. Moringa oleifera (Lam)-A nutritional powerhouse. J. Crop Weed 2017, 13, 238–246.
5. Patel B., Vekaria H. 23 Factorial design for optimization of HPLC-PDA method for the simultaneous estimation of Efonidipine Hydrochloride Ethanolate and

Telmisartan in Tablet Dosage Form. Journal for Basic Sciences. 22 (2022) (12): 1383-1392. DOI:10.37896/JBSV22.12/1673.
6. Fahey, J.W. Moringa oleifera: A review of the medicinal potential. Acta Hortic. 2017, 1158, 209–224.
7. Renityas, N.N. The Effectiveness of Moringa Leaves Extract and Cancun Point Massage Towards Breast Milk Volume on Breastfeeding Mothers. J. Ners Kebidanan (J. Nurs Midwifery) 2018, 5, 150–153.
8. T.R. Prashith Kekuda N. Mallikarjun, D. Swathi , K.V. Nayana ,Meera B Aiyar, T.R. Rohini. J. Pharm. Sci. & Res., 2010, 2(1), 34-37.
9. M.M. Rahman, M. M. Islam Sheikh, S. A. Sharmin, M. S. Islam, A. Rahman, M. Mizanur Rahman, M. F. Alam. CMU Journal, 2019, 8(2), 219-228.
10. K.V. Sashidhara , J.N. Rosaiah, E. Tyagi, R. Shukla, R. Raghubir, S.M. Rajendran. Eur J Med Chem., 209, 44(1), 432-436.
11. Depani JA, Chaudhary AB, Bhadani SM, Patel BD. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Bimatoprost and Timolol Maleate. World Journal of Pharmacy and Pharmaceutical Sciences. 7 (2018) (5), 741-750. DOI: 10.20959/wjpps20185-11452.
12. Bhoomi DP, Nidhi JD, Ankit C. Development and Validation of RP-HPLC Method for Estimation of Teneligliptin and its Impurity in Tablet. International Journal of Pharmaceutical Sciences Review and Research. 69 (2021) (2): 127-133. DOI:10.47583/ijpsrr.2021.v69i02.019
13. Patel BD, Vekaria HJ. Central Composite Design Expert-Supported RP-HPLC Optimization and Quantitative Evaluation of Efonidipine Hydrochloride Ethanolate & Chlorthalidone in Tablet. Journal of Chromatographic Science, 1 (2023) 8. DOI: https://doi.org/10.1093/chromsci/bmad077.
14. Patel DM, Chaudhary AB and Patel BD. Development and validation of RP-HPLC method for simultaneous estimation Beclomethasone Dipropionate, Phenylephrine Hydrochloride and Lignocaine Hydrochloride in cream. World Journal of Pharmacy and Pharmaceutical Sciences. 7 (2018) (5): 829-841. DOI: 10.47583/ijpsrr.2021.v69i02.019
15. Rajput S, Patel D, Rathi S, Patel B, Patel Y, Rajput S. Formulation and Evaluation of Rectal Insitu Gel of Phenylephrine for Treatment of Hemorrhoids. 23 (2023) (5): 790-802. DOI:10.37896/JBSV23.5/2155
16. Patel BD and Vekaria HJ. Quality by Design based method development and its validation for simultaneous estimation of Montelukast sodium and Bilastine in tablet dosage form. International Journal of Biology, Pharmacy and Allied Sciences. 11 (2022) (11) 5506-5516. DOI: https://doi.org/10.31032/IJBPAS/2022/11.11.6608.
17. Charmy P.D., Ankit B.C., Bhoomi D.P. Development and validation of RP-HPLC method for simultaneous estimation of Saxagliptin and Dapagliflozin. World J. pharm. Res. 2018, V. 7(8), P. 803-812. DOI: 10.20959/wjpps20185-11469
18. T. Rastogi, V. Bhutda, K. Moon, P.B. Aswar, S.S. Khadabadi. Asian J. Research Chem. 2019, 20(2), 181-182.
19. B.D.Patel, S.G.Rathi, A.M.Suthar, P.V.Dobariya. RSM-CCD coupled RP-HPLC optimization and quantitative evaluation of antihypertensive drugs in tablet. Azerbaijan chemical journal. 2024, 2, 26-35. DOI: doi.org/10.32737/0005-2531-2024-2-26-35
20. Cuomo, P.; Papaianni, M.; Fulgione, A.; Guerra, F.; Capparelli, R.; Medaglia, C. An innovative approach to control H. Pylori-induced persistent inflammation and colonization. Microorganisms 2020, 8, 1214.