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RESEARCH ARTICLE

REVISED New Discovery of Covid-19 Natural-Based Potential Antivirus Herbal Supplement Products from Pinang Yaki (*Areca vestiaria*) Extract: A Preliminary Study by Untargeted Metabolomic Profiling [version 2; peer review: 1 approved, 1 approved with reservations, 1 not approved]

Previously titled: New Discovery of Covid-19 Natural-Based Antivirus Herbal Supplement Products from Pinang Yaki (*Areca vestiaria*) Extract by Untargeted Metabolomic Profiling

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Abstract

Background: Pinang yaki has bioactive compounds that have potential as a new herbal supplement. A better understanding of the bioactive compounds of pinang yaki using untargeted metabolomic profiling studies will provide clearer insight into the health benefits of pinang yaki and in particular its potential for the therapy and prevention of Covid-19.

Methods: Fresh samples of pinang yaki (*Areca vestiaria*) are obtained from forests in North Sulawesi Province, Indonesia. Samples were used for untargeted metabolomics analysis by UPLC-MS.

Results: Based on an untargeted metabolomic profiling study of pinang yaki, 2504 compounds in ESI- and 2645 compounds in ESI+ were successfully obtained. After the analysis, 356 compounds in ESI- and 543 compounds in ESI+ were identified successfully. Major compounds Alpha-Chlorohydrin (PubChem ID: 7290) and Tagatose (PubChem ID: 439312) were found in ESI+ and ESI-

Open Peer Review

Approval Status

	1	2	3
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version 1 08 Oct 2021	 view	 view	

1. **Toto Sudargo** , Universitas Gadjah Mada, Yogyakarta, Indonesia
2. **Zanariah Hashim**, Universiti Teknologi Malaysia, Skudai, Malaysia

Discussion: The Top 10 metabolites from pinang yaki extract (ESI+) juga have been indicated in preventing SARS Cov2 infection and have exhibited good neuroprotective immunity. Benzothiazole (PubChem ID: 7222), L-isoleucine (PubChem ID: 6306), D-glucono-delta-lactone (PubChem ID: 736), Diethylpyrocarbonate (PubChem ID: 3051), Bis(2-Ethylhexyl) amine (PubChem ID: 7791), Cinnamic acid (PubChem ID: 444539), and Trigonelline (PubChem ID: 5570) also had potential effects as an antiviral, anti-inflammatory, and anti-Covid19.

Conclusion: Untargeted metabolomic profiling showed many bioactive compounds contained in pinang yaki (*Areca vestiaria*) extract. The top 10 compounds have been identified and explored for their potential benefits as anti-Covid19 supplement products. This is a preliminary study which still needs further research such as preclinical and clinical trials.

Keywords

Mass spectrometry-based metabolomics, natural-based antivirus, pinang yaki, *Areca vestiaria*, SARS-CoV-2, medicinal plants, functional food

This article is included in the **Plant Science** gateway.

3. **Nawaporn Vinayavekhin** , Chulalongkorn University, Bangkok, Thailand

Any reports and responses or comments on the article can be found at the end of the article.

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REVISED Amendments from Version 1

This article was revised according to reviews from reviewers which included changes to:

1. Title changed to: New Discovery of Covid-19 Natural-Based Potential Antivirus Herbal Supplement Products from Pinang Yaki (*Areca vestitaria*) Extract: A Preliminary Study by Untargeted Metabolomic Profiling.
2. Adding a sentence to the abstract conclusion: This is a preliminary study which still needs further research such as preclinical and clinical trials.
3. Adding the details of the method of providing samples.
4. A little grammar refinement.

Any further responses from the reviewers can be found at the end of the article

Introduction

Coronavirus disease 19 (COVID-19) is a highly communicable and deadly virus caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that first appeared in Wuhan, China in December 2019 and has since spread around the world (Mouffouk et al., 2021). The SARS-CoV-2 virus is a β -coronavirus, a non-segmented enveloped positive-sense RNA virus with a crown-like appearance and symmetric helical nucleocapsid (Astuti & Ysrafil, 2020). Unlike the previous outbreaks of coronavirus, SARS-CoV-2 is more transmittable and the majority of those infected remain asymptomatic, resulting in ineffective containment and mitigation (Andersen et al., 2020). It has become a major cause of mortality and morbidity worldwide (Das et al., 2021). Therefore, finding an optimal therapeutical solution is vital.

Medicinal plants and natural products can be promising alternatives to supplements or drugs to treat and prevent diseases (Benarba & Pandiella, 2020). Pinang yaki (*Areca vestitaria*), also known as crown shaft palm, is an endemic palm plant that grows in North Sulawesi that is traditionally utilized for the treatment of diabetes and diarrhoea, and as a contraceptive (Gosal et al., 2017; Herny et al., 2010). Pinang yaki fruit extract has bioactive compounds such as flavonoids, tannins, saponins, triterpenoids, and hydroquinones, which are known primarily as natural antioxidants (Simbala et al., 2017). Tannins and flavonoids are known for their antitumor, antiallergic, antihepatotoxic, and antioxidant activities (Londok et al., 2017), while triterpenoids exert antibacterial, anticancer, anti-inflammation, and wound care properties (Herny et al., 2010), as well as inhibiting viral replication (Das et al., 2021). Saponins also show antifungal, cytotoxic, antibacterial, and antiviral properties (Kregiel et al., 2017). A recent review also stated that flavonoids may inhibit viral replication and translation (Ahmad et al., 2015), enhance immune activity, antiviral protection, and reduce respiratory problems (Yang et al., 2020); since phenols inhibit the fusion of the virus to the host cell (Das et al., 2021).

Despite all those health-beneficial properties, pinang yaki is still underutilized. Pinang yaki and its bioactive compounds have the potential as a novel herbal supplement. A better understanding of pinang yaki's bioactive compounds using an untargeted metabolomic profiling study will provide clearer insight into the health benefits of pinang yaki, in particular its potential for therapy and prevention of Covid-19.

Methods

Fresh samples of pinang yaki (*Areca vestitaria*) fruit with the age of 3.5 months were obtained from Bolaang Mongondow forest in North Sulawesi Province, Indonesia. The samples were then cleaned using distilled water (Aquadex) and then stored in a cooling box to be sent to the intended metabolomic testing laboratory. Samples were used for untargeted metabolomics analysis by UPLC-MS. The botanical identification and authentication were confirmed at the Department of Pharmacology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Indonesia. Untargeted metabolomics analysis and identification of compounds were conducted at *Apical Scientific Sdn. Bhd.* 43300 Seri Kembangan laboratory, Selangor, Malaysia with registration number #CPMO08102001a.

Instruments and reagents

Ultimate 3000LC combined with Q Exactive MS (Thermo Fisher), Temp functional Centrifugation (Eppendorf), ACQUITY UPLC HSS T3 (100 × 2.1 mm × 1.8 μ m), Acetonitrile (Merck), Methanol (Merck), Formic acid (CNW).

Sample preparation

Samples were thawed, and 50 mg of each sample was precisely weighed into a tube, add with 800 μ L of 80% methanol with vortex for 90 s, and followed by sonication for 30 min, 4 °C. Then all samples were kept at −40 °C for 1 h. After that, samples were vortexed for 30 s, kept for 0.5 h, and centrifuged at 12000 rpm and 4 °C for 15 mins. Finally, 200 μ L of supernatant was transferred to a vial for LC-MS analysis.

Liquid chromatography-mass spectrometry (LC-MS)

Performed by Ultimate 3000LC combined with Q Exactive MS (Thermo Fisher) and screened with electrospray ionization-mass spectrometry (ESI-MS). The LC system is comprised of an ACQUITY UPLC HSS T3 (100 × 2.1 mm, 1.8 µm) with Ultimate 3000LC. The mobile phase is composed of solvent A (0.05% formic acid-water) and solvent B (acetonitrile) with a gradient elution (0-1.0 min, 95% A; 1.0-12.0 min, 95-5% A; 12.0-13.5 min, 5% A; 13.5-13.6 min, 5-95% A; 13.6-16.0 min, 95% A). The flow rate of the mobile phase is 0.3 mL·min⁻¹. The column temperature is maintained at 40 °C, and the sample manager temperature is set at 4 °C.

Mass spectrometry parameters in positive ion mode (ESI+) and negative ion mode (ESI-) mode are listed as follows:

- ESI+: Heater Temp 300 °C; Sheath Gas Flow rate, 45arb; Aux Gas Flow Rate, 15arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.0 KV; Capillary Temp, 350 °C; S -Lens RF Level, 30%.
- ESI-: Heater Temp 300 °C, Sheath Gas Flow rate, 45arb; Aux Gas Flow Rate, 15arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.2 KV; Capillary Temp, 350 °C; S -Lens RF Level, 60%.

Results

Graphs shown in [Figures 1 and 2](#) were used to determine the peak representing the number of annotated ions, retention time, and relative abundance of the ions. This data was then used to identify the compounds contained in the sample extract. The results of identification and mass of compounds were carried out by the laboratory of Apical Scientific Sdn. Bhd and the results provided in an Excel spreadsheet ([Microsoft Excel](#), RRID:SCR_016137) ([see data availability statement](#)) ([Nurkolis & Simbala, 2021](#)). Based on an untargeted metabolomic profiling study of pinang yaki, 2504 compounds in ESI- and 2645 compounds in ESI+ were successfully obtained ([Nurkolis & Simbala, 2021](#)). After the analysis, 356 compounds in ESI- and 543 compounds in ESI+ were identified successfully ([Nurkolis & Simbala, 2021](#)).

After 543 compounds in ESI+ were successfully identified, those compounds were ranked into the top 10 metabolites present in pinang yaki extract (ESI+), as is shown in [Table 1](#).

In [Table 2](#), the ranking is also shown for ESI-, providing the top 10 highest metabolite compounds. [Tables 1 and 2](#) show that chelidonic acid (PubChem ID: 7431) was present in both top 10 metabolites.

Figure 1. Total-Ion Current (TIC) was obtained from Pinang Yaki in ESI+ mode. Based on an untargeted metabolomic profiling study of pinang yaki, 2645 compounds in ESI+ were successfully obtained ([Nurkolis & Simbala, 2021](#)). After the analysis, 543 compounds in ESI+ were successfully identified ([Nurkolis & Simbala, 2021](#)). [Figure 1](#) shows that the major compound was Alpha-chlorohydrin (PubChem ID: 7290).

Figure 2. Total-Ion Current (TIC) was obtained from Pinang Yaki in ESI⁻ mode. In ESI⁻, a total of 2504 compounds were obtained, and 356 compounds were successfully identified by name (see underlying) (Nurkolis & Simbala, 2021). Figure 2 shows that the identified major compound was tagatose (PubChem ID: 439312).

Table 1. Top 10 metabolite from Pinang Yaki (ESI⁺).

Molecular weight (g/mol)	m/z	RT (min)	Name	Peak area	PubChem ID
103.09999	104.1073	0.818	Choline	24527.6	305
135.01414	136.0214	6.321	Benzothiazole	15776.47	7222
131.09455	132.1018	1.529	L-Isoleucine	15164.33	6306
110.01313	111.0204	14.528	Alpha-Chlorohydrin	14077.62	7290
178.04759	179.0549	0.876	D-Glucono-Delta-Lactone	12736.37	736
162.05267	163.0599	0.839	Diethylpyrocarbonate	10537.28	3051
241.27652	242.2838	6.909	Bis(2-ethylhexyl)amine	7771.887	7791
148.05225	149.0596	2.442	Cinnamic Acid	7221.059	444539
96.02104	138.0548	0.837	Trigonelline	6500.932	5570
184.00067	185.008	1.418	Chelidonic Acid	6135.752	7431

Table 2. Top 10 metabolite from Pinang Yaki (ESI⁻).

Molecular weight (g/mol)	m/z	RT (min)	Name	Peak area	PubChem ID
184.0002	182.9924	1.264	Chelidonic Acid	97389.49	7431
196.0573	195.05	0.799	Gluconic Acid	44676.74	10690
192.0261	191.0188	0.901	Citric Acid	21807.88	311
88.01464	87.0073	0.965	Pyruvic Acid	169834.1	1060
156.0181	155.0104	0.819	Propanediol 1-Phosphate	15028.76	440156
134.0201	133.0129	1.166	D-(+)-Malic Acid	14024.67	92824
166.9852	165.9779	6.659	2-Mercaptobenzothiazole	10616.37	697993
116.0096	115.0023	0.975	Maleic Acid	10462.28	444266
273.966	272.9587	0.698	1-O-Arsonopentofuranose	9882.362	25201247
180.0624	179.055	0.885	Tagatose	7052.94	439312

Discussion

The richness of Indonesia's natural ingredients and their active compounds still needs to be revealed and explored, in connection with the discoveries of new natural-based drug candidates. This untargeted metabolomic profiling study was conducted to clarify the content of compounds contained in pinang yaki (*Areca vestiaria*) and to see its potential as a Covid-19 herbal remedy. But of course, this study is a basic study that does not necessarily represent efficacy in animals (preclinical studies) and clinical trials in humans. However, the content of compounds that have been successfully identified in this study can be used as a basic reference in determining the dose of trials in animals (preclinical study).

Tables 1 and 2 show that chelidonic acid (PubChem ID: 7431) is in the top 10 metabolites using either ESI technique. A study conducted by Shin et al. (2011), showed the inhibitory effects of chelidonic acid on IL-6 production by blocking NF- κ B and caspase-1 in HMC-1 cells that can be a potential therapy for inflammatory diseases, including Covid-19 (Shin et al., 2011; Zhang et al., 2020). In addition, Tagatose (PubChem ID: 439312) which occupies the highest peak of ESI– mode (Figure 2), indicates that its presence in pinang Yaki (*Areca vestiaria*) is also quite high. Studies have shown that it can be a functional antidiabetic food for diabetes, which according to meta-analysis, is comorbid for Covid-19 patients (Guerrero-Wyss et al., 2018; Kun'ain et al., 2020). Alpha-chlorohydrin, also topped the highest position in ESI+ mode (Figure 1), which has a potential immunostaining effect in people with declining viral infections such as SARS Cov-2 (Soliman et al., 2014). However, there are negative effects of the use of Alpha-chlorohydrin on epididymis rats, therefore a comprehensive follow-up study is needed to look at the beneficial effects as well as their toxicity (Soliman et al., 2014).

In addition, choline (PubChem ID: 305) which is the Top 10 Metabolite from pinang yaki extract (ESI+) has also been widely researched for its effects in preventing SARS Cov-2 Infection and has good neuroprotective immunity (Chowdhury & Pathak., 2020). Other contents in pinang yaki (*Areca vestiaria*) such as Benzothiazole (PubChem ID: 7222) (Ali & Siddiqui., 2013), L-Isoleucine (PubChem ID: 6306) (Mao et al., 2018), D-Glucono-Delta-Lactone (PubChem ID: 736) (Kuwano et al., 2018), Diethylpyrocarbonate (PubChem ID: 3051) (Yamamura & Cochran., 1976), Bis(2-ethylhexyl) amine (PubChem ID: 7791) (Nazemi et al., 2014), Cinnamic acid (PubChem ID: 444539) (Gravina et al., 2011), and Trigonelline (PubChem ID: 5570) (Zhou et al., 2021) also have potential effects as an antiviral, anti-inflammatory, and anti-Covid19 agents.

Additionally, in ESI– mode, some of the highest-grade compounds in pinang yaki extract, such as gluconic acid (PubChem ID: 10690), citric acid (PubChem ID: 311), pyruvic acid (PubChem ID: 1060), propaediol 1-phosphate (PubChem ID: 440156), D-(+)-malic acid (PubChem ID: 92824), 2-mercaptobenzothiazole (PubChem ID: 697993), maleic acid (PubChem ID: 444266), and 1-O-arsonopentofuranose (PubChem ID: 25201247) have anti-inflammatory and antiviral effects.

The above compounds have been identified as contained in pinang yaki extract, which shows its potential as a Covid-19 antiviral herbal supplement product. Of course, it needs further study, researchers will conduct an *in vivo* or preclinical study to find out the effect of pinang yaki extract as an antiviral herbal supplement Covid-19 (based on the results of the compounds or metabolomic profiling in this paper).

Conclusion

This untargeted metabolomic profiling study shows many bioactive compounds are contained in pinang yaki (*Areca vestiaria*) extract. The top 10 compounds and their potential benefits as anti-Covid19 supplement products have been identified and explored.

Conflict of interest

Herni Emma Inonta Simbala, Fahrul Nurkolis, Nelly Mayulu, Linda Wilhelma Ancella Rotty confirming and declaring that all these authors have no conflict of interest.

Data availability

Underlying data

Figshare: Pinang Yaki (*Areca vestiaria*) Extract by Untargeted Metabolomic Profiling. <https://doi.org/10.6084/m9.figshare.16560276.v1> (Nurkolis & Simbala, 2021).

The project contains the following underlying data:

- Raw Data for Untargeted Metabolomic Profiling of Pinang Yaki (*Areca vestiaria*) Extract ESI+ and ESI–.

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

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In this manuscript, the authors identified metabolites in pinang yaki fruit extract using LC-MS and tried to link these metabolites to their anti-COVID19 properties. However, in my opinion, many points need to be improved before this manuscript can be indexed.

1. Title: The title does not represent accurately what the authors did. The word “untargeted metabolomics profiling” should be reserved for comparative studies. In addition, the authors did not perform experiments to show the relevance of these metabolites or even the pinang yaki itself on Covid-19 prevention or treatment at all, and thus, this should not be in the title. The appropriate title should be more like “Metabolite profiling of pinang yaki (*Areca vestiaria*) fruit” or something similar.
2. Introduction: The authors just mentioned the classes of metabolites in pinang yaki fruit, such as flavonoids, tannins, saponins, etc. As these classes of metabolites are quite common in plants, the statement did not give much information. Are there any known specific bioactive metabolites in pinang yaki fruit? If so, this should be mentioned.

Also, are there any known properties or benefits of pinang yaki fruit or extract at all?
3. The authors mentioned the use of UPLC-MS, but from the name of the instrument given, it's more like UHPLC system, rather than UPLC.
4. How exactly were the botanical identification and authentication carried out?
5. How many replicates of samples were carried out? I looked up the authors' raw data on the Excel spreadsheet, and the headings of the two columns are BH and PY. It's unclear what these abbreviations stand for. Are these the two replicates of the sample? In any case, more replicates are likely required to make sure that what the authors observed are not just some contaminants that got picked up by LC-MS.

6. Injection volume for LC-MS analysis should be specified.
7. The details of the data analysis method are needed. In addition, as metabolite identification is the main content of this report, it should be discussed in detail and should be done rigorously. How exactly were metabolites identified, i.e. by accurate mass, MS/MS, the use of standard, or the combination thereof? With the identification of tagatose, for example, how can you know it's not glucose or other sugars? From the structure, it should ionize better in the positive ion mode than in the negative ion mode as well, so it's a surprise that the authors did not observe a higher peak of tagatose in the positive ion mode.
8. Do the numbers of compounds detected in the positive and negative ion modes include isotopes and various adducts? How could you distinguish these from real compounds?
9. It's unclear how metabolites are ranked. Is it by peak area? However, the peak area in ESI-MS does not correlate with the abundance of metabolites, as different compounds have differing abilities to ionize in different modes. Thus, the levels of these different metabolites cannot be compared simply by peak areas. In addition, the statement that chelidonic acid could be found in the top 10 metabolites lists in both modes just means that it possesses the structure that allows it to ionize very well in both modes. It does not have any significance other than this.

If the authors want to really investigate the abundance of these metabolites in the sample, they will have to acquire the standard for each compound, and compare its abundance to the constructed standard curve.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: LC-MS-based metabolomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 28 February 2022

<https://doi.org/10.5256/f1000research.121189.r123459>

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Toto Sudargo

Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

I don't have any further comments.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Health and Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 31 January 2022

<https://doi.org/10.5256/f1000research.77431.r118669>

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Zanariah Hashim

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1. Please check the grammar. Specific names require capitalization (for example, department of pharmacology, faculty of mathematics and natural sciences, should be changed to Department of Pharmacology, Faculty of Mathematics and Natural Sciences), whereas compound names do not need to be capitalized (e.g. Chelidonic Acid --> chelidonic acid).
2. Which part of the plant was used as the extract? How old is the plant? Include more specifically where the plant comes from (not just some forests in North Sulawesi). Additionally, the authors need to explain the sample preparation procedure more clearly (including cutting, washing, sorting, etc.)
3. Explain how the compound identification was performed. This is the most vital part of this work.
4. Ref in Figure 1: please cite correctly: Nurkolis & Simbala.
5. What does "quantity" in Table 1 and Table 2 refer to? Is it peak area or peak height? Higher peak area/height does not necessarily mean that the compound is more abundant in the sample. It simply means the compound is easier (more sensitive) to be detected using the method. To confirm the abundance/concentration of the compound, the authors need to analyze standard compounds and draw calibration curves.
6. This is a preliminary result of untargeted metabolomic analysis of pinang yaki extract (which part should be mentioned clearly). Although the discovered compounds may have the potential to treat Covid-19, the discussion is based only on literature, without evidence from the authors themselves. As such, I think some parts are overly-discussed and the title is also misleading. Discussion can be improved by adding pathway analysis or other multivariate analysis.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: metabolomics, LC-MS, multivariate analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 29 October 2021

<https://doi.org/10.5256/f1000research.77431.r96491>

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Toto Sudargo

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1. The discussion should be more expressive. The contribution of this study to the literature should be highlighted.
2. Please ensure the manuscript is proofread and edited as there is a significant number of grammatical errors throughout the manuscript.
3. The abstract description is good, there is no need to rewrite to provide details. The most important result obtained from the study should be emphasized in this section.
4. Conclusion: Please rewrite. It should not be the summary of the findings as this has been

stated in the Results. The conclusion should be more concise and match the aim.

It can be seen from the research, that this research gives us new insights into the world of science, especially on the problem of Covid-19.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Health and Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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