REVIEW

The Potential Use of Bitter Leaf (*Vernonia amygdalina*) in Coronavirus Diseases (COVID-19)

Herbal Medicine Research Centre
Institute for Medical Research
National Institutes of Health
Ministry of Health Malaysia

Date of Report: 4th April 2020

Report Written By:	**Reviewed By:**
1. Terence Tan Yew Chin 2. Lim Xin Yi 3. Raja Nazatul Izni Raja Shahriman Shah 4. June Chelyn Lee	Dr. Ami Fazlin Syed Mohamed Head of Centre

Herbal Medicine Research Centre (HMRC)
Institute for Medical Research
Level 5, Block C6,
National Institutes of Health Complex
No. 1, Jalan Setia Murni U13/52, Section U13
40170 Setia Alam, Selangor

Disclosure

The authors of this review have no competing interest in this subject.

Disclaimer

This review is essentially a brief report, prepared on an urgent basis, to reflect the highest level of evidence available regarding the subject at this specific time. The conclusion draws on restricted reviews from analysis of pertinent literature, on expert opinion and/or regulatory status where appropriate. All efforts have been made to ensure all relevant published material has been reviewed but this document may still not fully reflect all scientific research available. Additionally, other relevant scientific findings may have been reported since completion of this review.

TABLE OF CONTENTS

Executive Summary

Title

The Potential Use of Bitter Leaf (*Vernonia amygdalina*) in Coronavirus Disease (COVID-19)

Objective

The objective of this report is to assess current available evidence on the potential of bitter leaf (*Vernonia amygdalina*) in COVID-19 management based on the following:

- Efficacy: focusing on bitter leaf reported properties of 1: antiviral, 2: modulation of immune response including anti-inflammatory, and 3: role as other supportive therapy or management of COVID-19; and their respective potential mechanism(s) of actions.
- Safety of bitter leaf

Methodology

Electronic databases were searched using pre-determined terminologies such as 'vernonia amygdalina', 'antiviral', immunomodulatory', 'immune response', 'inflammation', 'mechanism of action', and 'safety'. All clinical and preclinical studies (both in vitro and in vivo) related to safety and efficacy or effectiveness of bitter leaf in in treating viral diseases were included.

Results & Discussion

- There are no documented direct antiviral effects of *Vernonia amygdalina*.
- The *V. amygdalina* demonstrated differential and contrasting anti-inflammatory and immune enhancing effects in several preclinical studies of different inflammatory models such as bacterial infection, diabetes, and joint inflammation.
- Synergism between chloroquine and *V. amygdalina* has been reported for malaria but there are still no documented benefits of this combination for treatment of COVID-19 or any viral and respiratory infection.
- In terms of safety, *V. amygdalina* leaf extract is generally safe as aqueous and acetone extract but caution should be practiced in ethanol extracts as toxicity has been reported on testicular toxicity at 300 mg and 600 mg/kg and another paper showed low LD_{50} of 289 mg/kg compared to aqueous extract (LD_{50} > 5,000 mg/kg).

Conclusion

Bitter leaf (*Vernonia amygdalina*) demonstrated differential and contrasting anti-inflammatory and immune enhancing effects in several preclinical studies of different inflammatory models, such as bacterial infection, diabetes, and joint inflammation. There is no evidence of direct antiviral properties. In humans, given together with antiretroviral drugs, a single study demonstrated immune enhancing effects of *V. amygdalina* aqueous leaf extract in increasing CD4+ cell counts. It is important to identify the single and combination of various bioactive compounds found in *V. amygdalina* that may contribute towards reported differential anti-inflammatory (e.g. reduction of IL-6) and immune enhancing effects (as adjunct to vaccination), before further investigating their potential to be applied specifically in COVID-19 management.

Even though synergism between chloroquine and *V. amygdalina* has been reported for malaria, at present, there are still no documented benefits of this combination for treatment of COVID-19 or any viral and respiratory infection. Potential of synergism may be explored in future investigations, keeping in mind that as the combination may potentiate efficacy, it may also increase toxicity. Toxicology studies on the specific combination formula is essential.

Although consumption of *V. amygdalina* is generally considered to be safe in humans, additional safety considerations should be given on the type of extract used as ethanol extract has shown toxicity at 300 and 600 mg/kg in animal studies.

Full report on bitter leaf potential in COVID-19 management

Introduction

Bitter leaf or *Vernonia amygdalina* is traditionally used to alleviate boils and shingles, relieve stomach ache, stomach indigestion, liver problem, postpartum, and alleviate gout. (1) Decoctions of the leaves have also been used traditionally to alleviate fever, diarrhoea, dysentery, and cough as well as being used as a laxative. The leaves were also reportedly used for scabies and headache in Africa. (2)

Objective

The objective of this report is to assess current available evidence on the potential of bitter leaf in COVID-19 management based on the following:

- Efficacy: focusing on *Vernonia amygdalina* reported properties of 1: antiviral, 2: modulation of immune response including anti-inflammatory, and 3: role as

other supportive therapy for management of COVID-19; and their respective potential mechanism(s) of actions.

- Safety of *Vernonia amygdalina*

Methodology

Searching

Electronic databases were searched using pre-determined terminologies such as '*Vernonia amygdalina*', 'antiviral', 'immunomodulatory', 'immune response', 'mechanism of action', 'inflammation', and 'safety'. Any peer-reviewed journals found were included, which covers PubMed, Ovid Medline(R), EBM Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Cochrane database of systematic reviews. The articles included in the search strategy were limited to those which were published within the year 2007 to March 2020. All clinical and preclinical studies (both *in vitro* and *in vivo)* that evaluated *Vernonia amygdalina* in any antiviral, modulation of immune response, and safety studies were included.

Selection

All published articles related to safety and efficacy or effectiveness of *Vernonia amygdalina* in treating viral diseases were included.

Results & Discussion

Efficacy

Antiviral effects:

- No documented direct antiviral effects.

Anti-inflammatory effects:

- A 50% aqueous ethanol *V. amygdalina* leaf extract administered orally in post-menopausal osteoarthritis model of female Sprague Dawley (OVX-OA) rats demonstrated anti-inflammatory effects. The extract (150 mg/kg and 300 mg/kg) significantly down-regulated the inflammatory prostaglandin E2, nuclear factor κβ, IL-1β, ADAMTS-5, collagen type 10α1, and caspase3 in the OVX-OA rats. It up-regulated the anti-inflammatory cytokine interleukin (IL)-10 (at 300 mg/kg) mRNA expressions. (3)

- Ethanol extracts of young (EthYL) and old (EthOL) leaves of *V. amygdalina* (50-200 mg/kg) administered orally and diclofenac (10 mg/kg) were evaluated for anti-inflammatory activity in carrageenan-induced inflammation model in

rats. A dose-dependent increase in anti-inflammatory (demonstrated via reduction of paw oedema), antipyretic, and antinociceptive properties were observed in both EthYL and EthOL, similar to the standard drug (diclofenac). Mast cell degranulation accompanied by vasodilatation and high leukocytosis were observed in the negative control, but were markedly low in extract treated groups. EthYL of *V. amygdalina* was found to be more potent than EthOL. The anti-inflammatory mechanisms were unclear. (4)

- Methanol extract of *V. amygdalina* leaf (MEVA) administered orally was evaluated for anti-inflammatory effect in carrageenan hind paw oedema and carrageenan air pouch mice models. MEVA (50, 100, and 200 mg/kg) showed dose-dependent inhibition of oedema (41.4, 63.0, and 68.6%) at 4 h post-carrageenan injection. In the carrageenan air pouch model, MEVA (200 mg/kg) significantly ($p < 0.05$) reduced infiltrating leukocytes, protein concentration, and malondialdehyde (MDA) levels, while glutathione (GSH) and superoxide dismutase (SOD) were unaffected. The histological study showed a reduction in the infiltration of inflammatory cells in MEVA-treated groups. (5)

- Ethanol extract of the plant leaf administered orally (100 mg/kg, 300 mg/kg, and 1,000 mg/kg)for three days in Swiss mice infected with *Plasmodium berghei* demonstrated a dose dependent decrease in pro-inflammatory cytokines, serum interferon gamma (IFN-γ), and serum tumor necrosis factor-alpha (TNF-α) in contrast to the non-treated infected mice (6).

- Isolated vernoniosides A-D, four new Delta-7, 9(11)-stigmastane-type steroid saponins from the dried leaves *V. amygdalina* exhibited anti-inflammatory properties *in vitro* on nitric oxide (NO) production in lipopolysaccharide-activated murine macrophage cell line (7).

- Acetone leaf extract of *V. amygdalina* (100 mg/kg and 200 mg/kg; single dose) administered orally demonstrated anti-inflammatory effects comparable to indomethacin (10 mg/kg) via significant reductions in rats with oedema induced by both carrageenan and histamine. Potential mechanisms proposed include inhibition of prostaglandin release, though no detailed investigation was further conducted. (8)

- Ethanolic extract of *V. amygdalina* leaves given prophylactically for rats infected with *Staphylococcus aureus* was shown to effectively reduce the pro-inflammatory cytokine, interleukin (IL)-6 levels at a dose of 40g/200mg body weight (oral; three times daily). However, the extract also reduced the levels

of anti-inflammatory cytokine, IL-10. No bioactive compound was objectively measured in the serum and correlated to the outcomes though it was hypothesised that luteolin and myricetin were among the potential compounds to have contributed. (9)

- A patent (CN106038650A) was filed by a herbal company from Hubei, China for a preparation method and anti-inflammatory application of '*Vernonia amygdalina* Del. ethanol extract active ingredients'. The '*V. amygdalina* Del. ethanol extract active ingredients' were obtained by crushing *V. amygdalina* Del. leaves and then performing ethanol extraction. The '*V. amygdalina* Del. ethanol extract active ingredients' demonstrated anti-inflammatory activities of reducing C-reactive protein (CRP), IL-1β, IL-6, IL-8, and TNF-α cytokines of animal models with acute inflammation of the joints. (10)

Immunomodulatory effects:

- Aqueous extract of *V. amygdalina* leaves given orally demonstrated dose dependent (50 mg/kg to 800 mg/kg) positive effects on increasing CD4+ and white blood cells count in brown rats (*Rattus norvegicus*). The water-soluble fraction of the extract contained most of the phyto-constituents of the extract and thin layer chromatographic analysis of the fraction revealed the presence of fructo-oligosaccharide and galacto-oligosaccharide prebiotic though no statistical analysis to correlate compound content with observed effects was performed; while the mechanisms involved was also unclear. (11)

- Aqueous *V. amygdalina* extract administered orally in mice was suggested as an effective adjuvant to increase efficiency and reduce the number of required doses for hepatitis B vaccination. At a concentration of 250 mg/kg body weight as an adjuvant in a three times vaccination schedule, it increased IgM, IgG1 and IgA antibody responses. In a 2-times vaccination schedule, 1000 mg/kg of *V. amygdalina* as an adjuvant to hepatitis B vaccine was able to elicit effective antibody production (0.174±0.002) significantly ($p < 0.05$) higher than the conventional hepatitis B vaccine group (0.109±0.002) which received 3-times vaccine dose. It equally enhanced innate cell-mediated immune response by increasing total white blood cell, neutrophil and lymphocyte counts. The adjuvant-vaccine combination did not produce side effects as the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were within the normal ranges. (12)

- Aqueous extract of *V. amygdalina* leaves at 200 mg/kg, 400 mg/kg, and 800 mg/kg administered orally for 21 days was shown to significantly increase CD4+ cell counts in a dose dependent manner, compared to control, in

diabetic rats, suggesting immune boosting activities. Mechanisms of actions remained unclear as it was not investigated. (13)

- In contrast to the study by Momoh et al. 2010, another study found that 80% ethanol extract of *V. amygdalina* leaves (200 mg/kg, twice a day) administered orally for 21 days reduced CD4+ cell counts in diabetic and non-diabetic albino Wistar rats, suggesting immunosuppressive effects instead. There were no significant changes ($p > 0.05$) in other haematological parameters, including white cell counts. (14)

- The effects of ethanolic extract of *V. amygdalina* leaves (250 mg/mL) administered orally to Swiss albino rats (120-250 g) infected with *Clostridium sporogenes* on the immune system was investigated. A non-significant reduction in total white blood cell counts was observed in rats treated with the extract, compared to control. (16)

- A prospective, randomised, double-blinded, placebo-controlled clinical trial was conducted on 40 HIV-infected patients (10 patients in each group) to determine the effect of aqueous extract of *V. amygdalina* leaves alone, immunace capsules supplement alone, combination of aqueous extract of *V. amygdalina* and immunace capsule and a control group only on antiretroviral therapy. Results showed that administration of aqueous extract of *V. amygdalina* leaves or immunace capsules alone resulted in slight increase of CD4 count (4% and 5.6% respectively). However, administration of aqueous extract of *V. amygdalina* leaves in combination with immunace capsule showed significant ($p < 0.05$) greater increase in CD4 count (12%), which indicate a synergistic immunological effect of aqueous extract of *V. amygdalina* leaves in combination with immunace. However, aqueous extract of *V. amygdalina* leaves used in the study was prepared by soaking the fresh leaves (two handful) in 200 mL water and no chemical quantification of the components in the extracts was reported. In addition, the exact mechanism of action of how the plant affected the immune system has not been determined. (15)

Synergism with chloroquine:

- Aqueous extract of *V. amygdalina* leaf, administered orally in mice, was shown to act synergistically with chloroquine to produce enhanced antimalarial effects. Reduced parasite clearance time and recrudescent time with increased doses of *V. amygdalina extracts* (31.75 mg/kg, 62.5 mg/kg, and 125 mg/kg) in combination with chloroquine (10 mg/kg) (given for three days) was observed, irrespective of the resistance status of the parasite.

However, the mechanisms of synergism, whether it is pharmacokinetic or pharmacodynamic, remains unclear and requires further investigation. There were also concerns of additive negative effects resulting in more pronounced elevations in liver enzymes. (17)

- Chloroquine is one of the potential therapeutic agents being tested against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) currently. There is still ongoing debate as to whether chloroquine treatment is useful in COVID-19 patients, with some studies showing beneficial results (18,19) while one showed that it is not superior to control/non-treated patients (20). There are also concerns of potential adverse reactions in certain high risk groups and drug interactions. (19)

- A review paper which covers the medicinal properties, phytochemistry of different plant parts of *V. amygdalina*, pharmacology and toxicity. However, the references quoted for effect on CD4+ cell count (HIV/AIDS) was inaccurate as reference no.13 did not contain the details as mentioned and references quoted for toxicity was also inaccurate as reference no.89 was not about testicular toxicity. However, the correct references have been searched and reported in this document. (21)

<u>Safety</u>

- Aqueous extract of *V. amygdalina* dried leaves powder administered orally to male albino rats (130–150 g) once daily for five days showed no mortality with lethal dose at 50% (LD_{50}) > 5,000 mg/kg (22).

- Aqueous extract of *V. amygdalina* dried leaves powder (5,000 mg/kg) was administered orally to female Sprague dawley rats (250–300 g; 8–12 weeks old) at a single dose and were observed daily for 14 days. No mortality was observed throughout the study with (LD_{50} > 5,000 mg/kg). (23)

- Aqueous extract of *V. amygdalina* leaves (200, 400 and 600 mg/kg) was administered orally to 10 treatment groups (five females and five males Wistar rats each group (6–8 weeks old)) daily for a duration of 28 days. One death was observed at day 26 due to cannibalism caused by wounds. No pathological gross necropsy findings were detected and no significant findings on the hematological. Kidney congestion was observed in histopathology findings and no significant lesions were found in the liver, small intestines and heart. (24)

- Ethanolic extract of *V. amygdalina* leaves (100, 300, and 600 mg/kg)

administered orally to male Wistar rats showed safety at 100 mg/kg but demonstrated testicular toxicity at higher doses (300 and 600 mg/kg) (25).

- Aqueous oral extract of *V. amygdalina* in acute toxicity test in mice showed no mortality after 24h of treatment and the LD_{50} was greater than 5,000 mg/kg body weight. No death was recorded in all the groups throughout the 14 days monitoring period. It was also observed that the *V. amygdalina* extract induced a dose-dependent percent body weight change of 47.12% (for dose 10 mg/kg), 29.37% (100 mg/kg), 14.83% (1,000 mg/kg), 5.43% (1,600 mg/kg), 0.62 % (2,900 mg/kg) and 0.18% (5,000 mg/kg). No significant differences in organ weights were observed. (12)

- Acetone extract of *V. amygdalin* leaves administered orally (single dose; 100, 200, 400, 800, 1,600 and 3,200 mg/kg) did not show signs of acute toxicity, morbidity and mortality (8).

- Ethanol extract of *V. amygdalina* leaves administered intraperitoneally to mice showed no mortality with LD_{50} of 288.5 mg/kg. However, no full text is available to determine details such as mice species, details of the mice and duration of treatment. (26)

Conclusion

Bitter leaf (*Vernonia amygdalina*) demonstrated differential and contrasting anti-inflammatory and immune enhancing effects in several preclinical studies of different inflammatory models, such as bacterial infection, diabetes, and joint inflammation. There is no evidence of direct antiviral properties. In humans, given together with antiretroviral drugs, a single study demonstrated immune enhancing effects of *V. amygdalina* aqueous leaf extract in increasing CD4+ cell counts. It is important to identify the single and combination of various bioactive compounds found in *V. amygdalina* that may contribute towards reported differential anti-inflammatory (e.g. reduction of IL-6) and immune enhancing effects (as adjunct to vaccination), before further investigating their potential to be applied specifically in COVID-19 management.

Even though synergism between chloroquine and *V. amygdalina* has been reported for malaria, at present, there are still no documented benefits of this combination for treatment of COVID-19 or any viral and respiratory infection. Potential of synergism may be explored in future investigations, keeping in mind that as the combination may potentiate efficacy, it may also increase toxicity. Toxicology studies on the specific combination formula is essential.

Although consumption of *V. amygdalina* is generally considered to be safe in humans, additional safety considerations should be given on the type of extract used as ethanol extract has shown toxicity at 300 and 600 mg/kg in animal studies.

References

1. Mustapha NM, Nik Mahmood NZ, Mohd Ali NA, Haron N. Khazanah Perubatan Melayu. Tumbuhan Ubatan. Vol.II. Selangor: Institut Penyelidikan Perhutanan Malaysia. 2017:80–83.
2. Fomum FU. *Vernonia amygdalina* Delile. Wageningen, Netherlands: PROTA Plant Resources of Tropical Africa; 2004 [accessed 24 April 2015]. Available from: https://www.prota4u.org/database/protav8.asp?g=pe&p=Vernonia+amygdalina+Delile.
3. Madzuki IN, Lau SF, Abdullah R, Mohd Ishak NI, Mohamed S. Vernonia amygdalina inhibited osteoarthritis development by anti-inflammatory and anticollagenase pathways in cartilage explant and osteoarthritis- induced rat model. Phytotherapy Research. 2019;33(7):1784–1793.
4. Asante DB, Henneh IT, Acheampong DO, Kyei F, Adokoh CK, Ofori EG, et al. Anti-inflammatory, antinociceptive and antipyretic activity of young and old leaves of Vernonia amygdalina. Biomedicine & Pharmacotherapy. 2019; 111:1187–1203.
5. Onasanwo SA, Oyebanjo OT, Ajayi AM, Olubori MA. Anti-nociceptive and anti-inflammatory potentials of Vernonia amygdalina leaf extract via reductions of leucocyte migration and lipid peroxidation. Journal of Intercultural Ethnopharmacology. 2017;6(2):192–198.
6. Omoregie ES, Pal A. Antiplasmodial, antioxidant and immunomodulatory activities of ethanol extract of Vernonia amygdalina del. Leaf in Swiss mice. Avicenna Journal of Phytomedicine. 2016;6(2):236–247.
7. Quasie O, Zhang YM, Zhang HJ, Luo J, Kong LY. Four new steroid saponins with highly oxidized side chains from the leaves of Vernonia amygdalina. Phytochemistry Letters. 2016;15:16–20.
8. Adedapo AA, Aremu OJ, Oyagbemi AA. Anti-oxidant, anti-inflammatory and antinociceptive properties of the acetone leaf extract of Vernonia amygdalina in some laboratory animals. Advanced Pharmaceutical Bulletin. 2014;4(Suppl 2):591–598.
9. Setiawan LTK, Nugraha J, Lestari P, Sinnansari R, Soegiatro L, Handayani LPTM, et al. Indonesian Journal of Tropical and Infectious Disease. 2019;7(4):69–74.

10. Google patents: Anti-inflammatory application of *Vernonia amygdalina* Del. ethanol extract and preparation method thereof as well as preparation. [Internet]. [Accessed 7 April 2020]. Available at: https://patents.google.com/patent/CN106038650A/en
11. Im E, Ae A, Bn U, Po U. Immuno-modulatory properties of prebiotics extracted from Vernonia amygdalina. African Journal of Traditional, Complementary and Alternative. 2016;13(6):11–17.
12. Onah IA, Onuigbo EB, Odimegwu DC. Adjuvant effect of *Vernonia amygdalina* leaf extract on host immune response to hepatitis B virus subunit vaccine. Pharmazie. 2019 Mar 1;74(3):179–185.
13. Momoh M, Adikwu M, Oyi A. Vernonia amygdalina extract and CD4+ cell counts: an immune study. Global Journal of Biotechnology and Biochemistry Research. 2010; 5(2):92–96.
14. Eyong EU, Athangwho IJ, David-Oku E, Agiang MA, Ebong PE. Haematological and immunological effect of coadministration of extracts of Vernonia amygdalina and Azadirachta indica on normal and diabetic rats. African Journal of Biotechnology. 2011; 10(50):10258–10262.
15. Momoh MA, Muhamed U, Agboke AA, Akpabio EI, Osonwa UE. Immunological effect of aqueous extract of Vernonia amygdalina and a known immune booster called immunace(®) and their admixtures on HIV/AIDS clients: a comparative study. Asian Pacific Journal of Tropical Biomedicine. 2012;2(3):181–184.
16. Kola OM. Anti-Inflammatory activity of ethanolic leaf extract from Vernonia amygdalina on the immune system of Swiss albino rats dosed with Clostridium sporogenes. Research Journal of Medical Sciences. 2007;1:127–131.
17. Iwalokun BA. Enhanced antimalarial effects of chloroquine by aqueous Vernonia amygdalina leaf extract in mice infected with chloroquine resistant and sensitive Plasmodium berghei strains. African Health Sciences. 2008;8(1):25–35.
18. Touret F, de Lamballerie X. Of chloroquine and COVID-19. Antiviral Research. 2020 Mar 5;177:104762.
19. Yazdany Y, Kim AHJ. Use of hydroxychloroquine and chloroquine during the COVID-19 pandemic: what every clinician should know. Annals of Internal Medicine. 2020
20. Chen J, Liu D, Liu L. Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). Journal of Zhejiang University. 2020
21. Egharevba C, Osayemwenre E, Imieje V, Ahomafor J, Akunyuli C, Udu-Cosi AA, Theophilus O, et al. Significance of bitter leaf (Vernonia amagdalina) in tropical diseases and beyond: a review. Malaria Chemotherapy, Control & Elimination. 2014; 3: 120.
22. Oguwike FN, Offor CC, Onubeze DPM, Nwadioha AN. Evaluation of activities of bitterleaf (Veronia amygdalina) extract on haemostatic and biochemical profile of

induced male diabetic albino rats. IOSR Journal of Dental and Medical Sciences. 2013; 11(2):60–64.

23. Zakaria Y, Zainul Azlan N, Nik Hassan NF, Muhammad H. Phytochemicals and acute oral toxicity studies of the aqueous extract of Vernonia amygdalina from state of Malaysia. Journal of Medicinal Plants Studies. 2016; 4(3):1–5.
24. Nabukenya I., Rubaire-Akiiki C., Mugizi D., Katerengga J., Olila D, HÖglund J. Sub-acute toxicity of aqueous extracts of Tephrosia vogelii, Vernonia amygdalina and Senna occidentalis in rats. Natural Product Research. 2014:2(5):143.
25. Saalu LC, Akunna GG and Oyewopo A. The Histo-morphometric evidences of Vernonia amygdalina leaf extract-induced testicular toxicity. International Journal of Morphology. 2013; 31(2):662–667.
26. Ibrahim G, Abdurahman M, Ibrahim H, Ibrahim N, Magajii M (2011) Toxicity and analgesic effects of Vernonia amygdalina Del. (Asteraceae) leaf extract on mice. International Journal of Pharmacy and Biological Sciences. 1:1–4.