

# Lignans from *Morinda citrifolia* (Noni) Fruit Inhibit Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase *in Vitro*

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**How to cite this paper:** West, B.J., Deng, S.X. and Jensen, C.J. (2020) Lignans from *Morinda citrifolia* (Noni) Fruit Inhibit Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase *in Vitro*. *Journal of Biosciences and Medicines*, 8, 143-152.

<https://doi.org/10.4236/jbm.2020.83013>

**Received:** February 22, 2020

**Accepted:** March 10, 2020

**Published:** March 13, 2020

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## Abstract

*Morinda citrifolia* (noni) fruit juice has exhibited a variety of biological activities in human clinical trials, indicating that it influences multiple systems of the body. Since the 1990s, the endocannabinoid system (ECS) has been found to modulate the activity of other organ systems. To investigate noni's potential impact on the ECS, extracts from freeze-dried noni fruit were evaluated in fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibition assays. The ethyl acetate extract demonstrated the greatest activity against both enzymes. Lignans in this extract also inhibited enzyme activities, with americanin A being the most active in both assays. Americanoic acid and 3,3'-bisdemethylpinoresinol were the next most active compounds. These results suggest that lignans in noni fruit may influence endocannabinoid levels within the body via FAAH and MAGL inhibition. This reveals another set of probable mechanisms of action by which noni juice affects human health.

## Keywords

*Morinda citrifolia*, Noni, Endocannabinoid System, Fatty Acid Amide Hydrolase, Monoacylglycerol Lipase

## 1. Introduction

*Morinda citrifolia*, commonly known as noni, is a small to medium sized tree that grows throughout the tropics. This tree produces fruit year-round, which has a history of use as both food and medicine [1]. Noni leaves, flowers, bark and roots were also used in folk medicine among Pacific Islanders as well as in Southeast Asia and the Caribbean [2]. In fact, noni was reportedly the most im-

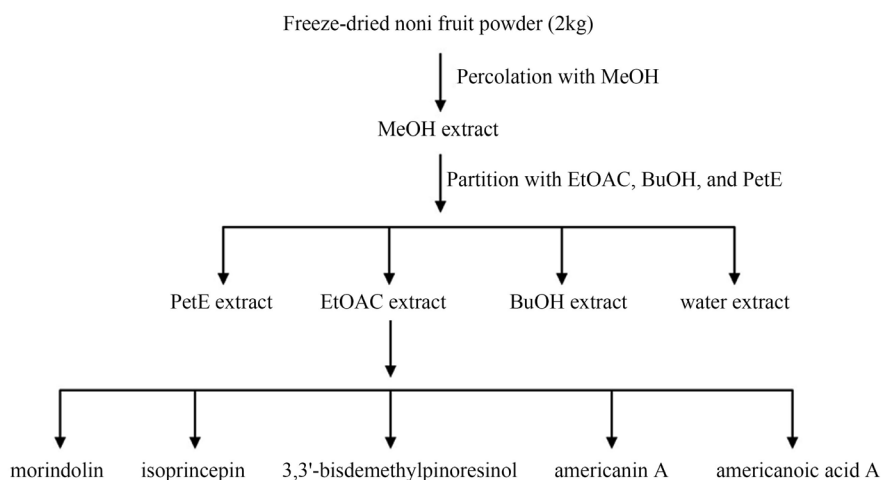
portant and widely used Polynesian medicinal plant prior to the European era [3]. Various parts of the plant were thought to be useful for a broad range of health conditions [4] [5] [6]. This perception appears to be supported by the results of human clinical trials, especially those involving noni fruit juice. Among the recorded effects of noni juice ingestion is control of inflammation, improved joint mobility, relief of discomfort, immune system modulation, protection against oxidative damage, maintenance of bone health, and improved mental health outcomes [7].

The wide array of noni juice health effects suggests that it interacts with multiple systems within the body, including the ECS. The ECS is involved in the regulation of the nervous, immune, digestive, reproductive, respiratory, renal, endocrine and cardiovascular systems [8] [9] [10] [11]. The ECS is composed principally of G protein-coupled receptors (GPCRs), lipid signaling molecules (endocannabinoids) and the enzymes responsible for endocannabinoid synthesis and catabolism. Among the GPCRs, the most prominent ECS receptors are cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2). But other GPCRs, or orphan receptors, that interact with endocannabinoids have also been discovered. There are two main endocannabinoids produced by the body, N-arachidonylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). AEA and 2-AG are synthesized from arachidonic acid derivatives, N-arachidonoyl phosphatidylethanolamine (NAPE) and diacylglycerol, by phospholipases and diacylglycerol lipases. AEA is eventually degraded by fatty acid amide hydrolase (FAAH) to arachidonic acid and ethanolamine. 2-AG is hydrolyzed by monoacylglycerol lipase (MAGL) and FAAH [12]. AEA and 2-AG are CB1 and CB2 ligands, and interactions between these produce physiological effects [13].

We previously reported that noni juice exhibits CB2 receptor agonist activity, indicating that some phytochemical constituents of noni fruit may exert an influence on the ECS via CB2 receptor binding [14]. We also discovered that processed noni fruit juice inhibits FAAH and MAGL *in vitro* [15]. Therefore, we sought to further investigate the interactions of various phytochemical constituents of noni fruit with FAAH and MAGL.

## 2. Materials and Methods

Noni fruit was harvested in French Polynesia and allowed to fully ripen. The fruit was then processed into a puree by mechanical removal of the seeds and skin, followed by pasteurization at a good manufacturing certified fruit processing facility in Mataiea, Tahiti. The puree was then freeze-dried. Twenty liters of methanol was percolated through 2 kg of the freeze-dried fruit puree powder. The methanol (MeOH) extract was concentrated via evaporation of the solvent. The MeOH extract was then diluted with 3 L water. Next, this was sequentially partitioned with 3 L petroleum ether (PetE) four times, 3 L ethyl acetate (EtOAc) three times, and 2 L butanol (BuOH) three times to yield corresponding extracts (Figure 1). The extracts were dried *in vacuo* with a rotary



**Figure 1.** Flow chart of the fractionation and isolation of noni fruit extracts and lignan compounds evaluated in FAAH and MAGL inhibition assays.

evaporator. The aqueous mother liquid was lyophilized to produce a dried water extract. Previously, the EtOAc extract was subjected to flash column chromatography, Sephadex LH-20, and reversed-phase preparative HPLC chromatography to yield several lignan compounds. Their chemical structures were elucidated by a series of spectroscopic techniques, including UV, IR, 1D and 2D NMR, as well as high resolution mass spectrometry. Several of these lignans displayed significant cyclooxygenase (COX) and lipoxygenase (LOX) inhibiting activity [16]. Other COX inhibitors have also been found to influence endocannabinoid catabolism [17]. Therefore, we focused on lignans from noni fruit in our assays, including morindolin, isoprincepin, 3,3'-bisdemethylpinoresinol, americanin A, americanoic acid A.

FAAH and MAGL inhibition assays were carried out as previously described, but with some modifications [18]. Human recombinant FAAH, human recombinant MAGL, 7-amino-4-methyl coumarin-arachidonamide (AMC-AA), and 4-nitrophenylacetate were obtained from Cayman Chemical Company (Ann Arbor, MI, USA). In the FAAH inhibition assay, the recombinant FAAH was diluted with Tris-HCl buffer (125 mM, pH 9.0) containing 1 mM EDTA. Noni fruit extracts were dissolved in DMSO with a final assay concentration of 500 µg/mL. Ten µL of each dissolved extract, or DMSO alone (vehicle control) were added to separate wells of a black plastic 96-well microplate. JZL195, was also included in the assay as a positive control. Inhibitor (samples and positive control) wells received 10 µL FAAH solution as well as additional 170 µL buffer. Background fluorescence wells, to which no FAAH was added, received 180 µL buffer. The microplate was incubated for five minutes at 37°C. Ten µL of AMC-AA solution was then added to each well with a final concentration of 20 µM. The microplate was then incubated again at 37°C for 30 minutes. Following incubation, the fluorescence intensity of each well was measured with a Synergy™ HT microplate reader (BioTek Instruments, Winooski, VT, USA) with 360 ± 40 nm excitation and 460 ± 40 nm emission. Fluorescence intensities (FI) were

corrected by subtracting background well values from those of corresponding sample wells. Percent FAAH inhibition was calculated from the difference between the fluorescence intensities of the vehicle control (no inhibition) and the sample, divided by that of the vehicle control alone.

$$\% \text{ FAAH inhibition} = 100 \times (\text{FI control} - \text{FI sample}) / \text{FI control}$$

In the MAGL assay, recombinant MAGL was diluted with Tris-HCl buffer (10 mM, pH 7.2) containing 1 mM EDTA. Extracts were dissolved in DMSO, and 10  $\mu\text{L}$  of each solution was added to separate wells of a clear plastic 96-well microplate. Again, the final concentration of each extract in the assay was 500  $\mu\text{g}/\text{mL}$ . A positive control, JZL195, was also included in separate wells. Vehicle control (no extract or positive control) wells received only DMSO. Sample and positive control wells received 10  $\mu\text{L}$  MAGL solution as well as additional 150  $\mu\text{L}$  buffer. The background absorbance wells, with no MAGL, received 160  $\mu\text{L}$  buffer. The wells were then incubated for 5 min. at room temperature. To each well, 10  $\mu\text{L}$  of 4-nitrophenylacetate in ethanol (236  $\mu\text{M}$  in final reaction solution) was added to begin enzymatic reactions. These were then incubated again at room temperature for 10 minutes. Afterwards, absorbance at 405 nm was then read with an ELX800 microplate reader (BioTek Instruments, Winooski, VT, USA). Absorbance values (A) were corrected by subtracting background well values from those of corresponding sample wells. Percent MAGL inhibition was calculated from the difference between control and sample absorbances divided by the absorbance of the control alone, as shown below.

$$\% \text{ MAGL inhibition} = 100 \times (\text{A control} - \text{A sample}) / \text{A control}$$

Averages and standard deviations, expressed as mean  $\pm$  standard deviation, were calculated for replicate analyses of the samples and positive control in both the FAAH and MAGL inhibition assays. These assays were conducted in 2019 at the research and development laboratory of New Age Beverages in American Fork, Utah.

### 3. Results and Discussion

All noni extracts inhibited FAAH activity to a greater degree than MAGL activity (**Table 1**). Against FAAH, the EtOAc, PetE and water extracts were the most active. But the EtOAc extract had the greatest potency, causing  $85.5\% \pm 10.9\%$  inhibition. This extract was also the most active extract in the MAGL assay. The PetE and the BuOH extracts modestly reduced MAGL function, but the MeOH and the water extracts were essentially inactive.

Since the EtOAc extract was the most effective against both enzymes, its phytochemical constituents were selected for further evaluation. We previously found that the EtOAc extract contains lignans that are biologically active against eicosanoid metabolizing enzymes. Therefore, we chose to assess the influence of five lignans in noni fruit on endocannabinoid hydrolysis enzymes.

Each lignan reduced both FAAH and MAGL activity (**Table 2**). However, three of the lignans had greater effects on MAGL. While all compounds reduced the

**Table 1.** Percent inhibition (mean  $\pm$  standard deviation) of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) by noni fruit extracts (500  $\mu\text{g}/\text{mL}$ ).

Extract	% Inhibition	
	FAAH	MAGL
MeOH	32.6 $\pm$ 9.2	9.8 $\pm$ 4.5
EtOAc	85.5 $\pm$ 10.9	49.0 $\pm$ 2.1
BuOH	32.6 $\pm$ 3.8	22.2 $\pm$ 1.4
PetE	69.6 $\pm$ 7.5	37.0 $\pm$ 0.7
Water	60.4 $\pm$ 7.5	4.1 $\pm$ 2.1
JZL195	80.4 $\pm$ 3.8*	81.8 $\pm$ 0.6**

\*JZL195 (positive control) tested at 50 nM, \*\*JZL-195 tested at 4  $\mu\text{M}$ .

**Table 2.** Percent inhibition (mean  $\pm$  standard deviation) of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) by lignans (200  $\mu\text{M}$ ) in noni fruit.

Compound	% Inhibition	
	FAAH	MAGL
morindolin	52.2 $\pm$ 3.8	36.8 $\pm$ 2.6
isoprincepin	47.8 $\pm$ 6.5	54.7 $\pm$ 0.5
3,3'-bisdemethylpinoresinol	71.7 $\pm$ 7.5	52.4 $\pm$ 6.0
americanin A	78.3 $\pm$ 3.8	82.8 $\pm$ 4.2
americanoic acid	58.7 $\pm$ 10.0	70.3 $\pm$ 4.1

function of at least one enzyme by more than 50%, americanin A was the most potent. It inhibited MAGL and FAAH by  $82.8 \pm 4.2$  and  $78.3\% \pm 3.8\%$ , respectively. The next most active lignans were americanoic acid and 3,3'-bisdemethylpinoresinol, with the former being slightly more active than the latter. It is interesting to note that while americanin A has a relatively equal effect on FAAH and MAGL, americanoic acid seems to be more selective against MAGL. Conversely, 3,3'-bisdemethylpinoresinol appears to be more selective against FAAH. While not as active as americanin A, isoprincepin also provides relatively balanced inhibition of both enzymes. Morindolin is more notable for its effect on FAAH.

Endocannabinoids accumulate at sites of injury and inflammation where they interact with cannabinoid receptors, transient receptor potential vanilloid 1 (TRPV1) ion channels and G protein-coupled GPR55 receptors to produce analgesia and anti-inflammatory effects [19] [20] [21]. Therefore, maintaining endocannabinoid tone, or levels, may produce analgesia. This possibility is demonstrated in the case of a 66-year-old female patient with lifelong history of pain insensitivity, rapid wound healing, and high stress tolerance [22]. The apparent cause was a single nucleotide polymorphism in FAAH that resulted in loss of enzyme function and elevated AEA signally. Additionally, URB597, a selective FAAH inhibitor, reduces neuropathic pain in Sprague-Dawley rats. But this analgesic ef-

fect is reduced when CB1 receptor and peroxisome proliferator-activated receptor alpha (PPAR alpha) antagonists are administered before URB597 [23]. MAGL inhibitors significantly reduce allodynia from chronic sciatic nerve constriction [24]. The dual FAAH/MAGL inhibitor JZL195 also reduces allodynia, motor incoordination, and catalepsy in C57BL/6 mice subjected to chronic constriction injury [25]. Interestingly, JZL195 reduced neuropathic pain better than selective FAAH or MAGL inhibitors alone. This suggests that substances which increase both AEA and 2-AG levels are more effective in treating pain at much lower doses, thus reducing the potential for side effects. The dual inhibition of these enzymes may also help explain the observed analgesic properties of noni juice in human patients [26].

ECS signaling is an important factor in regulating fear, anxiety, and other forms of psychological stress [27]. The amygdala processes emotions including fear and anxiety [28]. CB1 receptors are expressed at high levels in the amygdala, and decreased activation of these receptors appears to reduce expression of fear and anxiety in animal studies [29] [30]. Both FAAH deficient mice and those treated with an FAAH inhibitor had elevated brain AEA levels which lead to reduced anxiety when in the elevated plus maze [31]. In humans, a FAAH polymorphism resulting in ineffective enzyme activity is associated with lower general anxiety levels and enhanced fear extinction [32]. Further, 2-AG signalling appears to mitigate adverse effects of stress exposure, and the disruption of its synthesis impairs normal fear extinction in mice as well as increases anxiety-related behaviour [33] [34].

Noni juice improved the performance of male ICR mice, when compared to controls, in a Morris water maze (MWM) test following chronic restraint stress (CRS), as well as prevented stress-induced reduction in blood vessel density in the dentate gyrus of the hippocampus [35]. CRS increases FAAH activity and lowers AEA concentrations in the amygdala, increases anxiety and causes changes in amygdalar structure of C57/Bl6 mice. But none of these occur when FAAH deficient mice are subjected to CRS [36]. Further, the performance of MAGL knockout mice in hippocampus-dependent learning paradigms reveals that 2-AG signaling is important for learning and memory [3]. Therefore, FAAH and MAGL inhibition may be one of the mechanisms by which noni juice improved MWM performance.

We have found, for the first time, that lignans in noni fruit inhibit FAAH and MAGL activity. Only one lignan glycoside, pinoresinol 4-O- $\beta$ -d-glucopyranoside, was previously reported to inhibit MAGL activity [37]. Rather, lignans are better known for their antioxidant, antitumor, anti-inflammatory, antiviral, antimicrobial, and neuroprotective activities [38]. In fact, the most active lignan in our assays, americanin A, exhibits antioxidant, antibacterial, and anti-tumor properties. It also inhibits melanin synthesis and matrix metalloproteinase-1 expression and enhances choline acetyltransferase activity [39]-[47]. We now know that lignans, especially those in noni fruit, may also influence endocannabinoid metabolism.

## 4. Conclusion

Our findings reveal that lignans represent a new class of FAAH and MAGL inhibitors. Further, noni fruit juice ingestion may prolong AEA and 2-AG interactions with CB1, CB2, and orphan G-protein receptors. As such, noni juice might be helpful in conditions associated with insufficient endocannabinoid tone. This possibility should be evaluated *in vivo* and in human clinical trials. Additional lignan compounds should also be evaluated for their abilities to interact with the ECS via FAAH and MAGL inhibition.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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