Chemical Composition and Anti-Inflammatory and Antioxidant Activities of Extracts from Cultivated Morel Mushrooms, Species of Genus *Morchella* (Ascomycota)

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ABSTRACT: Species of genus *Morchella* are high-value edible mushrooms. They are sought after by culinary experts due to their aroma, flavor, meaty texture, and health benefits. *M. rufobrunnea*, *M. sextelata*, and *M. americana* were chosen in this study and investigated for their medicinal quality by using *in vitro* anti-inflammatory and antioxidant assays. This sampling represents conditions by which morels are produced (cultivated indoors, cultivated outdoors, and collected from natural habitats, respectively) for commercial markets. Both aqueous and methanolic extracts of all three morel species showed identical chromatographic and bioassay profiles, independent of their phylogenetic position or production method. In an antioxidant assay, aqueous and methanolic extracts of these mushrooms at 100 µg/mL inhibited lipid peroxidation (LPO) by 59%–62% and 33%–36%, respectively. In an anti-inflammatory assay using cyclooxygenase enzymes (COX-1 and COX-2), aqueous and methanolic extracts at 100 µg/mL showed COX-1 enzyme inhibition by 53%–57% and 30%–32% and COX-2 enzyme inhibition by 38%–44% and 16%–17%, respectively. Chromatographic purification and spectroscopic characterization of *M. rufobrunnea* extracts afforded five sugars (compounds 1–5), seven organic acids (compounds 6–13), three flavonoids (compounds 14–16), triglycerides, free fatty acids, and three sterols (compounds 17–19). This is the first report of COX-1 and COX-2 enzymes and LPO inhibitory activities of pure isolates (S)-morelid (compound 6), glutamic acid (compound 9), and brassicasterol (compound 19). This study also showed inhibitions of COX-1 (by 84%, 33%, and 37%), COX-2 (by 47%, 11%, and 22%), and LPO (by 74%, 48%, and 35%), respectively, at 25 µg/mL.

KEY WORDS: antioxidant, anti-inflammatory, cyclooxygenase enzyme, extracts, lipid peroxidation, *Morchella americana*, *M. rufobrunnea*, *M. sextelata*, edible and medicinal mushrooms

ABBREVIATIONS: AIA, anti-inflammatory activity; **AOA**, antioxidant activity; **BHA**, butylated hydroxyanisole; **BHT**, butylated hydroxytoluene; **BMAE**, black morel aqueous extract; **BMME**, black morel methanolic extract; **COX**, cyclooxygenase; **GMAE**, gray morel aqueous extract; **GMHE**, gray morel hexane extract; **GMME**, gray morel methanolic extract; **ITS**, internal transcribed spacer; **LPO**, lipid peroxidation; **LUV**, large unilamellar vesicle; **NSAID**, nonsteroidal anti-inflammatory drug; **PAUP***, phylogenetic analysis using parsimony*; **RO**, reverse osmosis; **RPB2**, second largest subunit of ribonucleic acid polymerases II; **TBHQ**, t-butylhydroquinone; **YMAE**, yellow morel aqueous extract; **YMME**, yellow morel methanolic extract

I. INTRODUCTION

Edible mushrooms are well known for their numerous health and nutritional benefits.[1–4] Commonly known as morels, species of genus *Morchella* (Ascomycota, Pezizales) are among the most distinguished and unique mushrooms. *Morchella* mushrooms are commonly foraged and used in gourmet cuisine due to their unique aroma, delicate flavor, and meaty texture.[4] *Morchella* mushrooms are distributed throughout the Northern Hemisphere and can be found in fields and forested habitats across Europe, North America, and

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Asia.[5–7] *Morchella* fruiting bodies have also been widely used in folk medicines to treat excessive sputum, gastrointestinal disorder, and shortness of breath.[4]

In previous studies, fruiting bodies and mycelia of *Morchella* species exhibited antioxidant, anti-inflammatory, antimicrobial, antiviral, antiallergic, antidiabetic, antiatherogenic, nephroprotective, and hepatoprotective activities.[4,8] In addition, phytochemical investigations of *Morchella* fruiting bodies and mycelia extracts have identified several secondary metabolites, including amino acids, tocopherols, phenolic acids, sterols, free fatty acids, vitamins, 5′-nucleotides, and polysaccharides responsible for its medicinal and pharmacological effects.[4,7] Similarly, polysaccharides containing α- and β-glycosidic bonds isolated from mycelia of *M. sextelata* exhibited immune-modulating activities against murine RAE264.7 cell lines.[8]

This study aimed to compare the methanolic and aqueous extracts from *M. rufobrunnea* Guzmán & F. Tapia, *M. sextelata* M. Kuo, and *M. americana* Clowez & C. Matherly under wild, outdoor, and indoor cultivation environments for their anti-inflammatory and antioxidant effects.

Phylogenetic analyses have identified three lineages within the genus *Morchella*, which consist of a basal lineage represented by *M. rufobrunnea* (Rufobrunnea clade, gray morels), the Elata clade (black morels), and the Esculenta clade (yellow morels).[6] We hypothesized that medicinal effects would differ between the species, and they would be further impacted by the way the morels are grown. To test this hypothesis, fruiting bodies of freshly harvested morel species representing three clades were sequentially extracted. These included *M. rufobrunnea*, (Rufobrunnea clade) from an indoor cultivation environment, *M. sextelata* (Elata clade) from an outdoor cultivation environment, and *M. americana* (Esculenta clade) that was wild foraged. Thus, in addition to maximizing sampled phylogenetic diversity, we tested for differences in medicinal quality that may result from three different ways by which morels are produced for commercial markets. Specimens from these three morel species were then sequentially extracted with water and methanol to yield aqueous and methanolic extracts, respectively. Extracts and purified isolates were then evaluated for anti-inflammatory activity (AIA) and antioxidant activity (AOA) using cyclooxygenase enzymes (COX-1 and COX-2) and lipid peroxidation (LPO) inhibitory assays, respectively.[9,10]

II. MATERIALS AND METHODS

A. Mushroom Samples

For this research, we studied *Morchella* mushrooms that were cultivated in both indoor and outdoor environments as well as those that were wild foraged in local Michigan forests. *M. rufobrunnea* was cultivated on pasteurized composted leaf-pine bark (1:1 v/v) in controlled indoor growing environments at the facilities of Gourmet Mushrooms Inc. (Scottville, MI) as described previously by Longley et al.[11] and by Gourmet Mushrooms Inc. (2017, gray morel; https://www.mycopia.com). *Morchella* ascocarps were harvested 31 days postinduction just prior to ascosporogenesis. *M. sextelata* (black morel, collection BR113) was cultivated outdoors in a forested environment in Michigan following previously described approaches.[12] Briefly, spawn consisting of woodchip, oats, and lime was first generated from an isolate of *M. sextelata* containing both mating types. Spawn was then planted in rows of soil prepared under the canopy of a deciduous forest in November 2018, and then covered with black plastic sheeting. Nutrient bags composed of woodchip and oats (70:30) were fed to the morel mycelium once the mycelium colonized the soil, approximately 2 weeks after planting spawn. In late May 2019, morels emerged and were harvested from the rows. *M. americana* (yellow morel, collection BR107) fruiting bodies were wild foraged in deciduous forest habitats in Michigan in late May 2019. Pure isolates of BR113 and BR107 were obtained and maintained on malt extract agar.

B. Molecular Analysis

Subsequently, the internal transcribed spacer (ITS) rDNA region was PCR amplified with primers ITS1f and ITS4 and then sequenced to confirm species identity by comparing against the National Center for Biotechnology Information database. RPB2 (second largest subunit of ribonucleic acid polymerases II) sequences were retrieved from GenBank, aligned with Mesquite (version 3.04), and analyzed with phylogenetic analysis using parsimony PAUP* (version 4.0a150) to reconstruct phylogenetic relationships, which were visualized in FigTree (version 1.4.4).

C. Extraction, Fractionation, and Purification

Fresh cultivated fruiting bodies of *M. rufobrunnea* (2.45 kg) were crushed into small pieces by hand and sequentially extracted with boiling reverse osmosis (RO) water (3 L, 2 h), methanol (2 L, 8 h × 3), and hexanes (2 L, 8 h × 3). Lyophilization of aqueous extract afforded gray morel aqueous extract (GMAE; 63.3 g). Evaporation of organic solvents under vacuum afforded gray morel methanolic extract (GMME; 20.1 g) and gray morel hexane extract (GMHE; 2.24 g). Stirring GMME with water (300 mL, 2 h) yielded water-soluble (GMME-1; 12.8 g) and water-insoluble (GMME-2; 7.3 g) fractions. Based on the identical thin-layer chromatography profiles, GMME-1 was combined with GMAE (3.11%) and GMME-2 was combined with GMME (0.39%).

Aliquots of outdoor-cultivated *M. sextelata* (340 g) and wild-harvested *M. americana* (yellow morel) (860 g) were sequentially extracted with boiling RO water (1.5 L, 2 h) and methanol (1.5 L, 8 h × 3). Lyophilization of aqueous extracts yielded *M. sextelata* aqueous extract (BMAE; 11.8 g, 3.47%) and *M. americana* aqueous extract (YMAE; 22.4 g, 2.60%). Evaporation of methanol under vacuum afforded black morel methanolic (BMME; 2.56 g, 0.75%) and *M. americana* methanolic (YMME; 4.73 g, 0.55%) extracts, respectively.

An aliquot of GMAE fraction (6.85 g) was fractionated on a CombiFlash Rf purification system and yielded subfractions A (5.56 g), B (1.77 g), and C (1.87 g), respectively (Fig. 1). An aliquot of fraction A (2.04 g) was further fractionated by a silica gel medium-pressure liquid chromatography purification system and yielded fractions A-1 (1.30 g), A-2 (66 mg), A-3 (220 mg), and A-4 (448 mg), respectively (Fig. 1). Purification of A-1 (200 mg) was accomplished by high-pressure liquid chromatography and yielded compounds **1** (22 mg), **2** (31 mg), **3** (130 mg), and **4** (16 mg), respectively (Figs. 1 and 2).[4] Similarly, purification of A-2 (60 mg) was also accomplished by high-pressure liquid chromatography and yielded compounds **5** (30 mg), **6** (16 mg), and **7** (12 mg), respectively (Figs. 1 and 2).[4,13] Purification of A-3 (40 mg) and A-4 (42 mg) by high-pressure liquid chromatography yielded compounds **8** (29 mg) and **9** (36 mg), respectively (Figs. 1 and 2).[4] Purification of fraction B (120 mg) by high-pressure liquid chromatography yielded compounds **10** (18 mg), **11** (21 mg), **12** (24 mg), and **13** (17 mg), respectively (Figs. 1 and 2).[14–17] Purification of fraction C (115 mg), accomplished by high-pressure liquid chromatography, yielded compounds **14** (40 mg), **15** (32 mg), and **16** (27 mg), respectively (Figs. 1 and 2).[10,18,19]

An aliquot of combined *M. rufobrunnea* methanolic extract (GMME; 1.61 g), fractionated by the CombiFlash Rf silica purification system, yielded fractions D (1.03 g), E (507 mg), and F (127 mg), respectively (Fig. 1). An aliquot of fraction D (615 mg), fractionated by the CombiFlash Rf silica gel medium-pressure liquid chromatography purification system, afforded fractions D-1 (420 mg) and D-2 (100 mg), respectively (Fig. 1). Purification of D-1 (40 mg) by preparative thin-layer chromatography gave a mixture of triglycerides (35 mg) (Fig. 1).[9] Similarly, an aliquot of D-2 (41 mg), also purified by preparative thin-layer chromatography, afforded a mixture of free fatty acids (32 mg) (Fig. 1).[9] An aliquot of fraction E (200 mg), fractionated by silica gel medium-pressure liquid chromatography, yielded fractions E-1 (104 mg) and E-2 (91 mg), respectively (Fig. 1). An aliquot of E-1 (35 mg) upon purification by preparative thin-layer

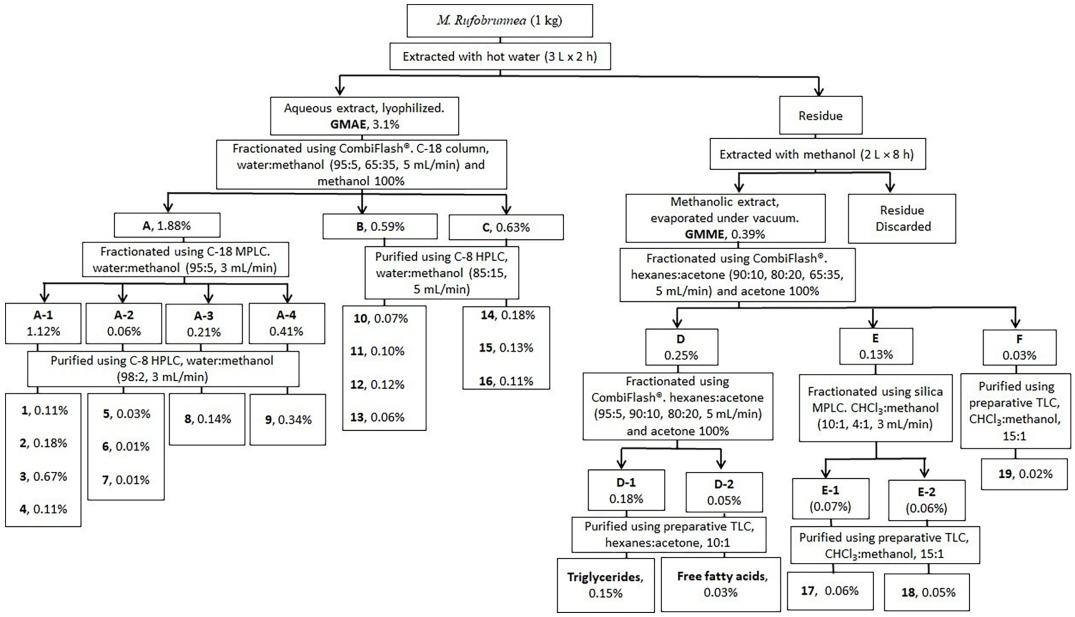

FIG. 1: Fractionation and purification of major compounds from *Morchella rufobrunnea* aqueous (GMAE) and methanolic (GMME) extracts. HPLC, high-pressure liquid chromatography; MPLC, medium-pressure liquid chromatography; TLC, thin-layer chromatography.

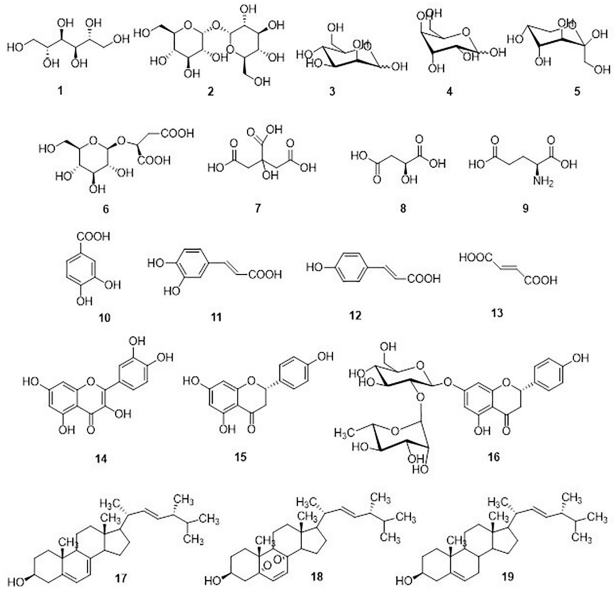

FIG. 2: Chemical structures of compounds (**1–19**) isolated from fruiting bodies of *Morchella rufobrunnea*. The identified compounds were confirmed by nuclear magnetic resonance spectral experiments: mannitol (**1**), trehalose (**2**), mannose (**3**), glucose (**4**), fructose (**5**), (S)-morelid (**6**), citric acid (**7**), malic acid (**8**), glutamic acid (**9**), protocatechuic acid (**10**), caffeic acid (**11**), *p*-coumaric acid (**12**), fumaric acid (**13**), quercetin (**14**), naringenin (**15**), naringin (**16**), ergosterol (**17**), ergosterol peroxide (**18**), and brassicasterol (**19**).

chromatography afforded compound **17** (28 mg) (Figs. 1 and 2).[18] Similarly, purification of an aliquot of E-2 (38 mg) by preparative thin-layer chromatography yielded compound **18** (29 mg) (Figs. 1 and 2).[18] Finally, purification of F (42 mg) by preparative thin-layer chromatography afforded compound **19** (36 mg) (Figs. 1 and 2).[4]

D. COX-1 and COX-2 Inhibitory Assays

The *in vitro* inhibition of two COX enzymes (COX-1 and COX-2) is an indication of AIA; hence, extracts from three aqueous (GMAE, BMAE, YMAE) and methanolic (GMME, BMME, YMME) extracts and purified isolates from *M. rufobrunnea* were investigated using this assay. COX-1 and COX-2 enzymes used in this study were prepared in our laboratory.[9,16,18] Commercial nonsteroidal anti-inflammatory drugs (NSAIDs) ibuprofen, naproxen, celecoxib (Celebrex; Viatris, Canonsburg, PA), and aspirin, as positive controls, were tested at 15, 12, 1, and 108 μg/mL, respectively. The varying concentrations of NSAIDs were used to afford inhibitory profiles between the range of 0% and 100%. Each sample was assayed in duplicate and the standard deviation was calculated for $n = 2$.

E. LPO Inhibitory Assay

To determine AOA, test samples were assayed for LPO inhibition by using large unilamellar vesicles (LUVs) according to procedures established in our laboratory.[10,16,18] The percent inhibition was calculated with respect to the dimethyl sulfoxide solvent control. Each sample was assayed in duplicate and the standard deviation was calculated for $n = 2$. The positive controls t-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were tested at 1.6, 1.8, and 2.2 μg/mL, respectively. Again, the varying concentrations of positive controls were used to obtain inhibitory profiles at the 0% and 100% range.

III. RESULTS

A. Structure Elucidation

The chemical structures of compounds **1–19** isolated from aqueous and methanolic extracts of *M. rufobrunnea* were elucidated by nuclear magnetic resonance (^{1}H and ^{13}C) spectral experiments and their identities were confirmed as mannitol (**1**), trehalose (**2**), mannose (**3**), glucose (**4**), fructose (**5**), (S)-morelid (**6**), citric acid (**7**), malic acid (**8**), glutamic acid (**9**), protocatechuic acid (**10**), caffeic acid (**11**), *p*-coumaric acid (**12**), fumaric acid (**13**), quercetin (**14**), naringenin (**15**), naringin (**16**), ergosterol (**17**), ergosterol peroxide (**18**), and brassicasterol (**19**), respectively (Fig. 2).[4,13–15,17–19] Since the compounds isolated were previously defined, a detailed spectral description of these compounds is not provided in this article.

Fraction D-1 was isolated as a colorless oil and identified as a mixture of triglycerides based on nuclear magnetic resonance (^{1}H and ^{13}C) chemical shifts.[9] The composition of the fatty acids in the triglyceride mixture was determined by comparison of retention times with retention times of authentic fatty acid methyl esters.[9] The retention times of 13.0, 13.9, 15.5, 15.6, and 16.1 min, respectively, suggested that the triglyceride mixture was esters of palmitic (16:0), palmitoleic (16:1), stearic (18:0) oleic (18:1), and linoleic (18:2) acids, respectively. Similarly, fraction D-2, isolated as a colorless oil, was identified as a mixture of free fatty acids based on characteristic nuclear magnetic resonance (^{1}H and ^{13}C) chemical shifts in its spectra.[9] The fatty acid composition in the mixture was determined by comparison of retention times with the retention time of authentic fatty acid methyl esters.[9] The retention times of 10.6, 13, 15.5, 15.6, and 16.1

min indicated that the mixture consisted of methyl esters of myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids, respectively.

B. AIA

COX enzymes catalyze the conversion of arachidonic acid to prostaglandins, thromboxanes, and other intermediates or inflammation-causing hormones that are partially responsible for the onset of inflammation in humans. Functional foods with the ability to inhibit COX enzymes can function as modulators of inflammation signaling pathways. Therefore, we tested the *in vitro* AIA of morel mushrooms by measuring COX-1 and COX-2 enzyme inhibition from its hot aqueous and methanolic extracts at 100 µg/mL according to published procedures.[9,16,18] Data from morel hot aqueous (GMAE, BMAE, YMAE) and methanolic (GMME, BMME, YMME) extracts showed COX-1 enzyme inhibition by 58%, 55%, 54%, 32%, 30%, and 31% and COX-2 inhibition by 41%, 44%, 39%, 16%, 17%, and 16%, respectively (Fig. 3). Commercial NSAIDs ibuprofen, naproxen, celecoxib, and aspirin were used as positive controls in this assay and tested at 15, 12, 1, and 108 µg/mL, respectively. They showed COX-1 enzyme inhibition by 56%, 59%, 27%, and 52% and COX-2 enzyme inhibition by 30%, 56%, 84%, and 35%, respectively (Fig. 3). Pure isolates **6**, **8**, and **13** at the 25 µg/mL concentration showed COX-1 enzyme inhibition by 84%, 21%, and 16% and COX-2 enzyme inhibition by 48%, 11%, and 14%, respectively (Fig. 3). A dose-response analysis of compounds **9** and **19** was carried out against COX-1 and COX-2 enzymes at 25, 50, 100, and 200 µg/mL concentrations. They inhibited COX-1 enzymes by 33%, 41%, 44%, 45%, 37%, 43%, 45%, and 45% and COX-2 enzymes by 12%, 22%, 26%, 27%, 23%, 28%, 29%, and 30%, respectively. We did not assay triglycerides, free fatty acids, organic acids (**7**, **10–12**), flavonoids (**14–16**), and sterols (**17** and **18**) in this study since their COX-1 and COX-2 enzyme inhibitory activities have been previously reported.[9,14–16,18] Sugars **1–5** and sugars in general do not show enzyme inhibitory activity and therefore were not investigated.

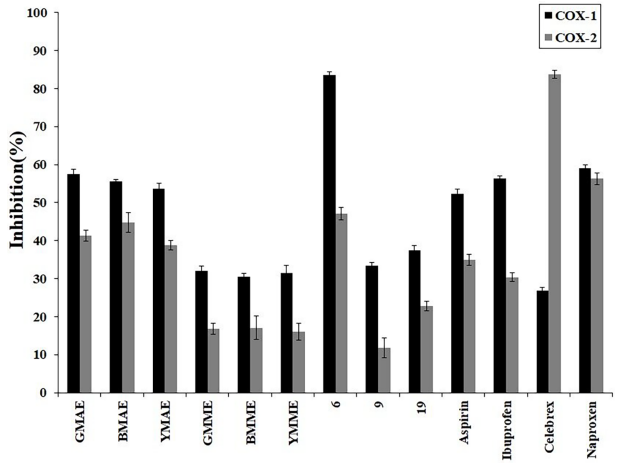

FIG. 3: COX-1 and COX-2 enzyme inhibitory activities of gray (GMAE), black (BMAE), and yellow (YMAE) morel aqueous and methanolic (GMME, BMME, YMME) extracts at 100 µg/mL. Purified isolates **6**, **9**, and **19** were tested at 25 µg/mL and commercial NSAIDs ibuprofen, naproxen, celecoxib, and aspirin at 15, 12, 1, and 108 µg/mL, respectively. Vertical bars represent the standard deviation of each data point ($n = 2$). The potency of COX enzyme inhibition varied with each NSAID. Therefore, the varying concentrations of positive controls were used to yield comparable inhibitory activity values between 0% and 100%.

C. AOA

To determine AOA, test samples were assayed for LPO inhibition by using LUVs according to procedures established in our laboratory.[10,16,18] The positive controls TBHQ, BHA, and BHT tested at 1.6, 1.8, and 2.2 µg/mL concentrations showed LPO inhibition by 87%, 88%, and 87%, respectively (Fig. 4). The morel hot aqueous extracts (GMAE, BMAE, and YMAE) exhibited AOA by 59%, 62%, and 61%, respectively, at 100 µg/mL concentration, compared with the activity of methanolic extracts (GMME, BMME, and YMME) by 36%, 33%, and 35%, respectively, at 100 µg/mL concentration (Fig. 4). Pure isolates **6**, **8**, **9**, **13**, and **19** exhibited LPO by 75%, 42%, 47%, 30%, and 35%, respectively, at 25 µg/mL concentration (Fig. 4). We did not assay sugars (**1–5**), triglycerides, free fatty acids, organic acids (**7**, **10–12**), flavonoids (**14–16**), and sterols (**17** and **18**) in this study since their LPO inhibitory activities have been reported.[9,14–16,18]

IV. DISCUSSION

In this study, fresh cultivated morel mushrooms of three *Morchella* species representing different phylogenetic lineages (*M. rufobrunnea*, the Rufobrunnea clade; *M. sextelata*, the Elata clade; and *M. americana*,

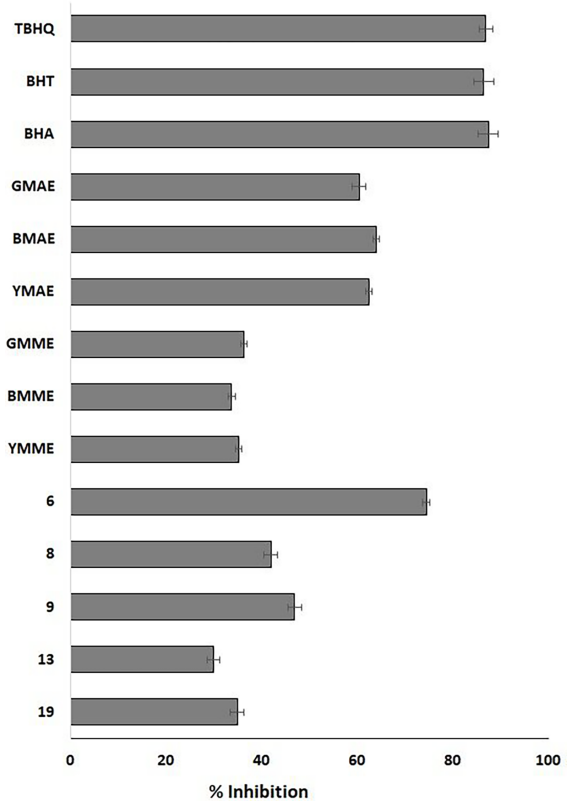

FIG. 4: LPO inhibitory activities of aqueous extracts of gray (GMAE), black (BMAE), and yellow (YMAE) and methanolic extracts of gray (GMME), black (BMME), and yellow (YMME) morel mushrooms tested at 100 µg/mL. Purified isolates **6**, **8**, **9**, **13**, and **19** were tested at 25 µg/mL and commercial antioxidants TBHQ, BHA, and BHT were tested at 1.6, 1.8, and 2.2 µg/mL, respectively. Horizontal bars represent the standard deviation of each data point (n = 2). The varying concentrations of positive controls and purified isolates were used to yield comparable inhibitory activity values between 0% and 100%.

the Esculenta clade) were evaluated for their chemical composition, AIA, and AOA using *in vitro* bioassays. These samples also represent the different ways that morels are procured for the commercial market, including indoor cultivation, outdoor cultivation, and wild foraging, which we expected would contribute to medicinal quality variation between the species, as determined through hot aqueous and methanolic extracts. The yields of total extracts varied among the tested morel species and were between 3.38% and 4.22% of fresh mushrooms. Among them, *M. sextelata* gave the highest percentage of total extract yield. Our previous work on edible mushrooms nameko (*Pholiota nameko*), shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus ostreatus*), and maitake (*Grifola frondosa*) yielded total extractable components between 6.3% and 12.7% of fresh mushroom weight.[9,20] With respect to other edible mushroom we have studied, morels contained significantly less extractable materials. Based on our investigation, the chromatographic profiles (CombiFlash Rf medium-pressure liquid chromatography and thin-layer chromatography) of aqueous and methanolic extracts from three different morel species studied were identical to each other. It is important to note that extracts from tested samples showed the presence of identical components, albeit with slight differences in their concentrations. That is, they all contained the same chemical compounds, with the difference being quantitative rather than qualitative. Our experience suggests that variation in secondary metabolites is quite common in biological materials procured from different locations and at different growing seasons. This result is particularly striking, given the deep phylogenetic relationships between the three species, in addition to the fact that the morels were grown in different substrates (cultivated versus wild foraged) and environments.

The AIA of tested extracts at 100 µg/mL determined by measuring the inhibition of the COX-1 and COX-2 enzymes to convert arachidonic acid to prostaglandin endoperoxide showed COX-1 enzyme inhibition by 53%–57% and 30%–32% and COX-2 enzyme inhibition by 38%–44% and 16%–17%, respectively, whereas NSAIDs ibuprofen, naproxen, celecoxib, and aspirin inhibited COX-1 by 56%, 59%, 27%, and 52% and COX-2 by 30%, 56%, 84%, and 35%, respectively (Fig. 3). Similarly, tested aqueous and methanolic extracts at 100 µg/mL were also evaluated for AOA using the LPO assay. The buildup of free radicals in the cell reacts with lipids, nucleic acids, proteins, and other vital cellular molecules. This reaction causes oxidative damage and stress at the cellular level, which in turn leads to numerous diseases including arthritis, cardiovascular diseases, and cancers.[21] The LPO assay used in this study measures the free radical scavenging capability of extracts and purified compounds according to procedures established by our laboratory.[10,16,18] The peroxidation of the lipid was initiated by the addition of Fe^{2+} resulting in the generation of reactive oxygen species, which cause the peroxidation of lipids and fluorescent probes embedded in the LUVs. The loss of fluorescent intensity in the assay indicates oxidative damage to the lipid substrate. Both extracts inhibited LPO by 59%–62% and 33%–36%, whereas they inhibited antioxidants TBHQ, BHA, and BHT by 87%, 88%, and 87%, respectively (Fig. 4). Biochemical similarities in the morels studied were further supported by the bioassay profiles of the *in vitro* AIA (Fig. 3) and AOA (Fig. 4) profile of aqueous and methanolic extracts. Hence, we focused on the purification and spectroscopic characterization of the major bioactive constituents of the hot aqueous and methanolic extracts of the *M. rufobrunnea*, for which we had the most biomass available.

Chromatographic purification of the crude hot aqueous extract of *M. rufobrunnea* afforded mannitol (**1**), trehalose (**2**), mannose (**3**), glucose (**4**), fructose (**5**), (S)-morelid (**6**), citric acid (**7**), malic acid (**8**), glutamic acid (**9**), protocatechuic acid (**10**), caffeic acid (**11**), *p*-coumaric acid (**12**), fumaric acid (**13**), quercetin (**14**), naringenin (**15**), and naringin (**16**) as major constituents and crude methanolic extract afforded triglycerides, free fatty acids, ergosterol (**17**), ergosterol peroxide (**18**), and brassicasterol (**19**) as the major constituents (Figs. 1 and 2).

Sugars (compounds **1–5**) were the most abundant metabolites in *Morchella* spp. studied and they constituted 1.1% of the total weight of the mushroom. These isolated sugars were not tested for the LPO and COX assays due to our prior knowledge of their lack of such biological activities. Organic

acids are the next most abundant class of secondary metabolites isolated from morels and amounted to 0.85% by fresh weight. Among the organic acids isolated, compound **6** (morelid) was reported from *M. deliciosa* Fr. and it exhibited an umami-like taste in trials.[13] In this work, we report the AIA and AOA of morelid for the first time. Morelid inhibited COX-1 and COX-2 enzymes by 84% and 48%, respectively, at a 25 μg/mL concentration and LPO by 75% at the same concentration (Figs. 3 and 4). Its COX enzyme inhibitory profile was similar to the inhibitory profile of aspirin and ibuprofen (Fig. 3). AOA of compounds **8**, **9**, and **13** was also reported for the first time and as indicated by the LPO inhibition of 42%, 47%, and 30%, respectively, at a 25 μg/mL concentration (Fig. 4). Compounds **8**, **9**, and **13** did not inhibit COX-1 and COX-2 enzymes significantly at 25 μg/mL. We previously reported the AOA of compounds **10**, **11**, and **12** and showed LPO inhibition by 90%, 88%, and 62% at 50, 5, and 10 μg/mL concentrations.[14–16] Compound **7** did not inhibit LPO. The organic acids **7**, **10**, and **12** showed little or no COX-1 and COX-2 enzyme inhibition and compound **12** selectively inhibited the COX-2 enzyme by 45% at 25 μg/mL.[14–16] Flavonoids are one of the important classes of fungal bioactive molecules that exhibit AOA and AIA. Isolation and spectroscopic characterization of three flavonoids in tested morels revealed 0.42% of morel fresh weight. The AOA and AIA of flavonoids **14–16** was previously reported.[21–23]

Triglycerides and free fatty acid fractions constituted up to 0.18% of the fresh weight of morel mushrooms. These fractions consisted of saturated fatty acids tetradecanoic (myristic, 14:0), hexadecenoic (palmitic, 16:0), and octadecanoic (steric, 18:0) acids and unsaturated fatty acids 9-cis-hexadecenoic (palmitoleic, 16:1), (9Z)-octadecenoic (oleic, 18:1), and cic,cis-9,12-octadecadienoic (linoleic, 18:2) acids. The various saturated and unsaturated fatty acids were previously evaluated for their LPO and COX activities.[24] Saturated fatty acids myristic (14:0) and palmitic (16:0) acids showed high LPO inhibitory activities, while unsaturated fatty acids showed significant COX enzyme inhibition.[24] The presence of phytosterols in *Morchella* was up to 0.13% of its fresh weight (Fig. 2). In our report on *Agrocybe aegerita*, compounds **17** and **18** at 100 μg/mL inhibited COX-1 enzyme by 19% and 57% and COX-2 enzyme by 28% and 22%, respectively.[25] Therefore, these compounds were not reevaluated in this study. Sterol **19** was assayed in this study and showed COX-1 and COX-2 enzyme inhibition by 38% and 23%, respectively, at 25 μg/mL. The COX enzyme inhibitory profile of this steroidal compound is similar to the inhibitory profile of aspirin (Fig. 3). Sterols **17** and **18** exhibited LPO inhibition by 45% and 43%, respectively, at 100 μg/mL. The LPO inhibition of sterol **19** is reported (Fig. 4).[25] It is interesting to note that the COX and LPO inhibitory activities of sterol **19** were superior to those of sterols **17** and **18**.

V. CONCLUSIONS

This study aimed to determine whether health benefits of *Morchella* differ between species or by the conditions under which they grow (wild foraged, indoor cultivated, and outdoor cultivated). *In vitro* anti-inflammatory (COX) and antioxidant (LPO) bioassays were used to assess the medicinal (AOA and AIA) value of *Morchella* mushrooms. In order to maximize both phylogenetic diversity, we chose to sample a diverse set of *Morchella* mushroom species grown with various production methods, including wild foraging and indoor and outdoor cultivation. Similar to plants, mushrooms grown under different environmental conditions have the potential to up- or downregulate the concentrations of secondary metabolites in their edible biomass that are beneficial to human health. Likewise, secondary metabolite production in mushroom fruiting bodies was expected to be dictated by the substrate in which they are grown. Yet we found that the secondary metabolite profiles between the species differed only in quantity rather than quality. It is important to note that extracts from the three different samples of morels showed the presence of identical components, albeit with slight differences in their concentrations. That is, they all contain the same chemical compounds, with differences being quantitative rather than qualitative. This is relevant because *M.*

rufobrunnea mushrooms produced indoors are currently available in the market, while morels produced in outdoor settings are expected to increase both the availability and affordability to consumers.

Morchella mushrooms were found to be rich sources of triglycerides, free fatty acids, sterols, sugars, organic acids, and flavonoids. The indoor-cultivated *M. rufobrunnea* fruiting bodies studied afforded 1.1% of sugars, 0.85% of organic acids, 0.42% of flavonoids, 0.18% of triglycerides and fatty acids, and 0.13% of sterols on a fresh weight basis. This may be considered as a similar range of concentrations of compounds present in other *Morchella* spp. used in this study. It is evident that *in vitro* AOA and AIA exhibited by crude extracts and the pure isolates of *Morchella* studied support the notion that indoor-cultivated *Morchella* mushrooms possess numerous health-beneficial properties in addition to providing regular nutrition and culinary taste.

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