ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Polyphenol-Rich Extracts of Xylopia and Aframomum Species Show Metabolic Benefits by Lowering Hepatic Lipid Accumulation in Diet-Induced Obese Mice

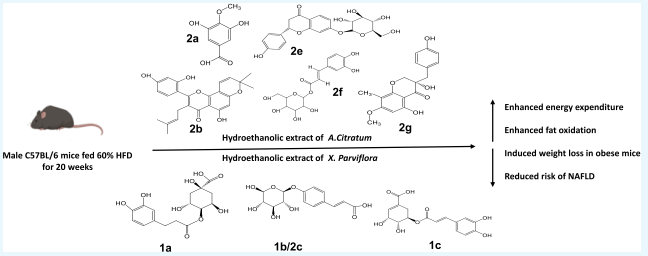
Achille Parfait Nwakiban Atchan, Shilpa Talkad Shivashankara, Stefano Piazza, Armelle Deutou Tchamgoue, Giangiacomo Beretta, Mario Dell'Agli, Paolo Magni, Gabriel Agbor Agbor, Jules-Roger Kuiaté, and Uma Venkateswaran Manjappara*

Cite This: *ACS Omega* 2022, 7, 11914–11928

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Metabolic syndrome is a complex condition associated with a series of pathologies featuring glucose intolerance, diabetes, high blood pressure, dyslipidemia, microalbuminuria, overweight, and obesity. It is also related to nonalcoholic fatty liver disease (NAFLD), recognized as the most familiar cause of chronic liver disease worldwide. The overall prevalence of metabolic syndrome and, consequently, the one of NAFLD is constantly increasing worldwide. The initial management of these diseases involves lifestyle modifications, including changes in diet and physical exercise. In addition to conventional drugs like orlistat, botanicals are traditionally used to counteract these disorders, and some of them are currently under evaluation. The present work evaluated the in vivo beneficial effects of hydroalcoholic extracts of two Cameroonian spices, focusing on obesity-related hepatic lipid injury in high-fat-fed C57BL/6 mice. Hydroethanolic extracts were prepared and characterized by reverse phase-high-performance liquid chromatography (HPLC)-photodiode array detection and ultra performance liquid chromatography-triple time-of-flight electrospray ionization tandem mass spectroscopy (TOF-ESI-MS/MS) analysis. Plant extracts were orally administered for 30 days at different dose levels (100 and 200 mg kg^{-1} body weight (BW)) to obese C57BL/6 mice. Food intake (FI) and BW were recorded daily. Plasma biochemical parameters and lipid content were estimated at the beginning and at the end of the experiment. Liver tissues were subjected to histological examinations, lipid content, as well as oxidative stress markers, and FAME (fatty acid methyl esters) were estimated. Oral administration of extracts at 200 mg kg^{-1} BW significantly reduced FI and prevented BW gain. A decrease in the weight of the liver and a decrease in the hepatic and plasma lipid content were observed. Plasma enzyme (serum glutamic-oxaloacetic transaminase, SGOT; serum glutamic pyruvic transaminase, SGPT; alkaline phosphatase, ALP) activities were not indicative of any organ damage. Chemical analysis suggested that phenolic acids (4-caffeoylquinic acid, p-coumaric acid 4-O-glucoside, 5-caffeoylshikimic acid, caffeic acid hexose, and 4-O-methyl gallic acid) and flavonoids (morusin derivatives, naringenin-7-O-glucoside, and homoisoflavanone) identified in the extracts could potentially justify the biological properties observed. The main findings of this study showed that *Xylopia parviflora (A. Rich.) Benth* and *Aframomum citratum (Pereira ex Oliv. et Hanb.) K. Shum* decreased hepatic lipid accumulation in high-fat-diet (HFD)-induced obese C57BL/6 mice and confirmed, at least in part, our previous in vitro and ex vivo studies. The molecular mechanisms underlying these effects are still unclear and will be explored in the future.



INTRODUCTION

The metabolic syndrome (MetS) is a constellation of different pathological features involving all or almost all organs and tissues. It is a cluster of risk factors that include abdominal obesity, hyperlipidemia, hyperglycemia, and hypertension. MetS have received a lot of interest because of the increasing association with cardiovascular morbidity and mortality.[1] The definition of MetS includes high blood pressure (BP), high blood glucose concentration, increased waist circumference (WC), high triglycerides (TG), and low high-density lipoprotein (HDL). The liver (along with the kidney) is frequently and heavily involved, with the onset of nonalcoholic fatty liver disease (NAFLD) actually recognized as the most familiar cause of chronic liver disease worldwide.[2] The estimated prevalence of NAFLD is thought to be around

Received: January 4, 2022
Accepted: March 17, 2022
Published: March 30, 2022

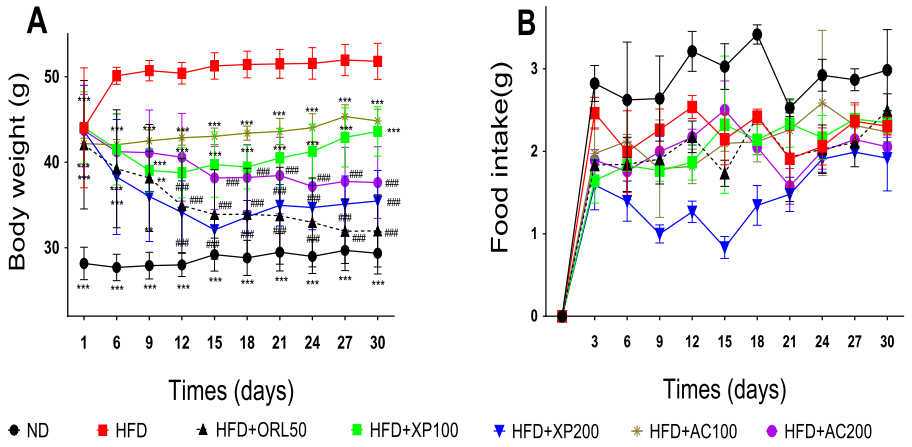


Figure 1. Effect of spice extracts on BW and FI in high-fat diet-induced obesity in C57BL/6 mice. (A) BW and (B) FI. Values represent the mean ± SD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs the HFD group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs ND group. ND, normal diet; HFD, high-fat diet; ORL, orlistat; XP, *Xylopia parviflora*; and AC, *Aframomum citratum*.

25−35% across the globe and affects different proportions of men and women. It is estimated that among the population suffering from NAFLD, 75% are obese individuals and even more are patients with type 2 diabetes mellitus (T2DM).[3] Most of the patients with NAFLD develop simple steatosis, but in up to one-third of patients, NAFLD progresses to its more severe form, nonalcoholic steatohepatitis (NASH), characterized by liver injury and inflammation (liver cirrhosis, hepatocarcinoma, and cardiovascular diseases).[4] Because NAFLD is closely associated with insulin resistance, obesity, hypertension, and dyslipidemia, it is regarded as the liver manifestation of MetS. Fatty infiltration of the liver results from increased hepatic lipid accumulation and, at the same time, decreased hepatic lipid clearance. In addition, it is well established that adipose tissue has a substantial impact on the accumulation of lipids in the liver, predominantly by the release of free fatty acid (FFA), proinflammatory cytokines, and induction of reactive oxygen species (ROS), which are considered as a critical pathological factor for NASH development and progression.[5]

Many pharmacological treatments are often prescribed [i.e., statins, pioglitazone, glucagon-like peptide-1-receptor agonists (GLP-1RAs)] to reduce hepatic liver accumulation; nevertheless, these drugs are only effective at the last stage of the disease and can additionally exert serious side effects that can lead to low therapy compliance.[6] Thus, especially in the initial phases of NAFLD, for which no specific drugs are available, the identification, validation, and availability of functional foods and food supplements able to reduce lipid accumulation in the liver may be welcome.

In addition, herbs/spices traditionally used to remedy a wide range of diseases have been systematically re-evaluated rather than conventional drugs. Our previous studies[7−9] clearly demonstrated that some selected edible plants from Cameroon positively modulate enzymes relevant to carbohydrate/lipid and cardiometabolic disorders. Furthermore, we clearly showed that on hepatic and adipose human cell models, those spice extracts inhibited ROS production and improved glucose uptake, reduced lipid storage, and exhibited anti-inflammatory activity.[8,10] Our first chemical profiling suggested that polyphenols could potentially justify the biological properties observed.[7] The present study evaluated the *Xylopia parviflora (A. Rich.) Benth* and *Aframomum citratum (Pereira ex Oliv. et Hanb.) K. Shum* hydroethanolic extracts for in vivo beneficial effects against hepatic injury induced by high-fat diet (HFD) in *C57BL/6* mice and further isolated and chemically characterized the most abundant polyphenols occurring in the extracts.

RESULTS

Effect of Hydroethanolic Spice Extracts on Cumulative FI, and Body and Tissue Weights in Diet-Induced Obese Mice. After feeding HFD to *C57BL/6* mice for 18 weeks, the body weight (BW) of the HFD groups increased by 37.24% (from 25.88 to 41.24 g) while the BW of the normal chow diet (ND) increased by 11.10% (from 24.26 to 27.29 g). During the 30 days treatment, HFD was provided every day to mice. Before administering the respective treatment (ORL50, XP100 and 200, AC100 and 200), BW and food intake (FI) were recorded. Compared with the mice fed with ND, the mice fed with HFD presented a significant ($p < 0.001$) increase in BW. Orlistat (tetrahydrolipstatin, administered to the mice at 50 mg/kg BW) and spice extracts at 200 mg/kg BW significantly ($p < 0.001$) reduced the BW compared with the mice-HFD group receiving saline (Figure 1A). On the contrary, mice administered 100 mg/kg BW of spice extracts did not show any significant ($p > 0.05$) changes in BW. No significant difference in FI between groups was observed (Figure 1B), and no signs of pathology or abnormalities were observed. Different organs, viz. the liver, kidney, spleen, heart, lungs, testis, and brain, were collected, weighed, and normalized to their respective BWs. As shown in supplementary Table 1S, there was a significant ($p < 0.01$) difference in liver weight between the ND-fed mice group and the HFD-fed mice group, both receiving saline. The liver of the treated groups had a significant ($p < 0.001$) lower weight than HFD-fed mice. Similar observations were shown on the heart weight of different groups. Moreover, there were no significant ($p > 0.05$) changes in the weight of the other organs.

Effect of Hydroethanolic Spice Extracts on Plasma Biochemical Parameters. Plasma biochemical profiles of all groups are shown in Table 1 and supplementary Table 2S. Glucose, triglycerides, total cholesterol, HDL cholesterol, and protein were estimated using standard kits (Agape Pharmaceutical, Kerala, India). At the beginning of the treatment, only glucose, total cholesterol, and HDL cholesterol levels were

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

Table 1. Effect of Spice Extracts on Glucose, Lipid, and Protein Plasma Biochemical Parameters in DIO-C57BL/6 Mice Post- and Pretreatment[a]

groups of mice		ND	HFD	HFD + ORL50	HFD + XP100	HFD + XP200	HFD + AC100	HFD + AC200
glucose (mg/dL)	AHFDIO	170.6 ± 10.17	240.9 ± 13.94*					
	A30DT	150.9 ± 14.33	243.7 ± 9.38***	191.10 ± 10.33$^{***\#\#\#}$	235.50 ± 15.41***	175 ± 7.90$^{\#\#\#}$	214.10 ± 14.35$^{*** \#\#}$	166.30 ± 16.30$^{\#\#\#}$
triglycerides (mg/dL)	AHFDIO	21.84 ± 2.11	22.39 ± 2.28					
	A30DT	31.96 ± 2.48	59.91 ± 8.37***	54.82 ± 8.45***	56.74 ± 11.96***	41.39 ± 8.75$^{\#}$	44.14 ± 3.42	42.03 ± 8.76$^{\#}$
total cholesterol (mg/dL)	AHFDIO	66.07 ± 7.33	179.40 ± 9.79***					
	A30DT	75.71 ± 7.50	192.80 ± 13.09***	147.60 ± 16.36$^{***\#\#\#}$	183.60 ± 15.77***	126.70 ± 14.83$^{***\#\#\#}$	147.60 ± 11.02$^{***\#\#\#}$	144.70 ± 17.48$^{***\#\#\#}$
HDL cholesterol (mg/dL)	AHFDIO	61.71 ± 6.29	142 ± 15.05***					
	A30DT	68.48 ± 5.25	147.80 ± 5.39***	110 ± 12.66$^{***\#\#\#}$	140.40 ± 16.95***	100.90 ± 10.82$^{***\#\#\#}$	137 ± 12.25***	117.20 ± 7.38$^{***\#\#\#}$
total protein (g/dL)	A30DT	5.63 ± 0.47	5.87 ± 0.63	5.98 ± 0.27	5.73 ± 0.32	5.53 ± 0.19	5.46 ± 0.22	5.98 ± 0.26

[a]The values represent the mean ± SD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs the HFD group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs ND group. HFDIO, high-fat-diet-induced obese; AHFDIO, after high-fat diet-induced obesity; A30DT, after 30 days of treatment; ND, normal diet; HFD, high-fat diet; ORL, orlistat; XP, *Xylopia parviflora*; and AC, *Aframomum citratum*.

significantly ($p < 0.001$) increased in the HFD-fed mice group compared to those in the ND-fed mice group (an increase of 29.18% of glucose, 63.17% of total cholesterol, and 56.69% of HDL cholesterol) (Table 1). When the ratio of HDL to total cholesterol was calculated, there was a significant reduction of 14−16% in the HDL/total cholesterol percentage in the HFD group compared to the ND group. No significant differences were seen between the HFD and treatment groups. In addition, at the end of the treatment, the glucose and lipid levels of the HFD-fed mice group were significantly ($p < 0.001$) higher than those in ND-fed mice (an increase of 38.07% of glucose, 46.65% of triglycerides, 60.73% of total cholesterol, and 53.66% of HDL cholesterol). The HFD-fed mice group treated with spice extracts at 200 mg/kg BW showed a significantly lower ($p < 0.001$) glucose level (28−31%) than the HFD-fed mice group. Treatment with orlistat (50 mg/kg BW) and spice extracts (100 mg/kg BW) significantly ($p < 0.01$) reduced the glucose level (12−21%) compared to the HFD group, and no significant ($p > 0.05$) difference was shown when compared with the ND group. Similar observations on the total cholesterol and HDL cholesterol levels were observed (Table 1). However, compared to the HFD group, treatment with spice extracts at 200 mg/kg BW significantly reduced ($p < 0.05$) the triglyceride level. Moreover, no significant ($p > 0.05$) difference in the plasma protein level was observed among the different groups (Table 1).

We estimated the level of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase enzymes, which are the indicators of liver damage, and measured the level of metabolites creatinine and urea to assess renal function (supplementary Table 2S). All the groups evinced different values of SGOT and SGPT, which were found to be in the normal range (reference ranges of SGOT and SGPT in plasma are, respectively, up to 46 and 49 U/L). Furthermore, the blood plasma analysis demonstrated that groups did not significantly differ in alkaline phosphatase enzymes, creatinine, and urea levels, indicating that the treatment does not damage the liver and kidney function.

Effect of Hydroethanolic Spice Extracts on Hepatic Lipid Accumulation. Liver weight abnormalities and hepatic steatosis are common obesity-related phenomena. Therefore, we measured the lipid content in the liver of all treated groups, as shown in Figure 2. The results showed significantly ($p < 0.001$) higher triglycerides and total cholesterol levels in the HFD group than the ND group (an increase of 84.02 and 76.08% of triglycerides and cholesterol content, respectively) (Figure 2A,B). Treatment with orlistat at 50 mg/kg BW and spice extracts at 200 mg/kg BW significantly ($p < 0.01$) reduced triglycerides and cholesterol content (50.41−82.86% triglycerides and 53.76−89.49% cholesterol content) compared with the HFD group. However, HFD-fed mice receiving spice extracts at 100 mg/kg BW showed a less significant difference than the HFD-fed mice group.

Effect of Hydroethanolic Spice Extracts on the Liver-FFA Profile. We performed a gas chromatography−mass spectrometry (GC−MS) analysis to study the liver-FFA profile of all the experimental groups (Table 2). We confirmed the presence of eleven (11) FFAs ranging from **C14:0** to **C20:4** in the HFD-fed mice group, where palmitic (**C16:0**) and oleic (**C18:1**) acids were predominant. Similar observations were shown in HFD-fed mice and treated with *A. citratum* extract at

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

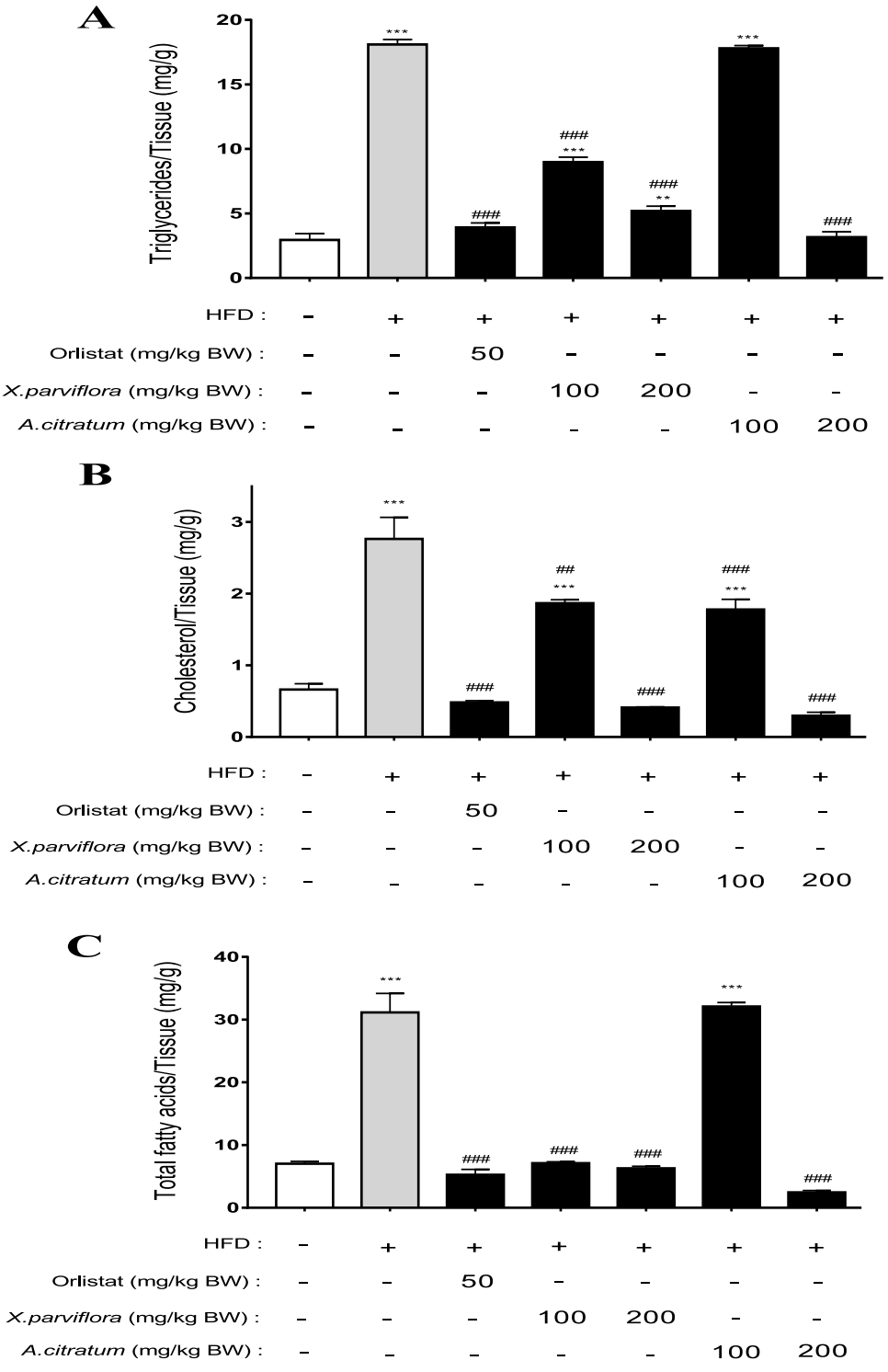


Figure 2. Effect of spice extracts on liver lipid contents. (A) Effect of spice extracts on triglyceride contents. (B) Effect of spice extracts on cholesterol contents. (C) Effect of spice extracts on the total FFA contents. Values represent the mean ± SD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs the HFD group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs the ND group. HFD, high-fat diet.

100 mg/kg BW. However, **C15:0** (pentadecylic), **C18:3** (linolenic: cis 9,12,15), **C20:1** (gondoic: cis 11), **C20:3** (dihomo-γ-linolenic: cis 7, 10, 13) fatty acids were not detected in ND-fed and HFD-fed groups, orlistat or *X. parviflora* extract (100 and 200 mg/kg BW), and *A. citratum* extract at 200 mg/kg BW. Only mice administered 200 mg/kg BW *X. parviflora* showed significantly ($p < 0.001$) low palmitic acid (23.19 ± 0.34% fatty acid) compared to the HFD-fed mice group. The total amount of FFAs was increased in the HFD-fed mice group compared to the ND group. However, HFD-fed mice groups showed a significant ($p < 0.001$) decrease in the total amount of FFAs (Figure 2C). Those FFAs were distributed in saturated and unsaturated fatty acids (Figure 3A). The HFD-fed mice group showed higher amounts of both saturated and unsaturated compared with the other groups. Those saturated and unsaturated FFA

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

Table 2. Fatty Acid Profile of Liver Fat from Different Experimental Groups after 30 Days of Treatment[a]

groups of mice	ND	HFD	HFD + ORL50	HFD + XP100	HFD + XP200	HFD + AC100	HFD + AC200
group of fatty acids	amount of fatty acids in one milligram of total fatty acid extracted from liver fat (%)						
C14:0	0.51 ± 0.00	0.66 ± 0.02	0.67 ± 0.00	0.54 ± 0.01	0.57 ± 0.03	0.50 ± 0.00	0.00
C15:0	0.00	0.08 ± 0.01	0.00	0.00	0.00	0.08 ± 0.00	0.00
C16:0	26.32 ± 1.11	28.12 ± 0.07	29.24 ± 0.90	28.45 ± 0.05	23.19 ± 0.34*###	27.66 ± 0.38	27.04 ± 0.39
C16:1	5.64 ± 0.20	5.30 ± 0.01	3.59 ± 0.04	4.04 ± 0.03	4.54 ± 0.01	3.84 ± 0.07	3.60 ± 0.23
C18:0	7.41 ± 0.57	4.92 ± 0.07	7.47 ± 0.00	4.75 ± 0.19	5.69 ± 0.23	5.86 ± 0.09	6.65 ± 0.11
C18:1	42.32 ± 2.54	42.45 ± 0.18	40.75 ± 1.09	42.04 ± 0.41	44.74 ± 0.47	39.11 ± 0.68*#	40.57 ± 0.68
C18:2	12.48 ± 0.46	13.07 ± 0.12	14.20 ± 0.20	15.49 ± 0.01*	15.19 ± 0.05	14.70 ± 0.15	15.69 ± 0.26
C18:3	0.00	0.24 ± 0.00	0.00	0.00	0.00	0.13 ± 0.00	0.00
C20:1	0.00	0.63 ± 0.00	0.00	0.00	0.00	0.54 ± 0.01	0.00
C20:3	0.00	0.49 ± 0.00	0.00	0.00	0.00	0.49 ± 0.02	0.00
C20:4	5.58 ± 0.18	3.99 ± 0.08	4.04 ± 0.04	4.63 ± 0.24	6.03 ± 0.01	7.03 ± 0.06#	6.42 ± 0.09

[a]The values represent the mean ± SD. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ vs the HFD group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs ND group. HFD, high-fat diet; ND, normal diet; HFD, high-fat diet; ORL, orlistat; XP, *Xylopia parviflora*; and AC, *Aframomum citratum*.

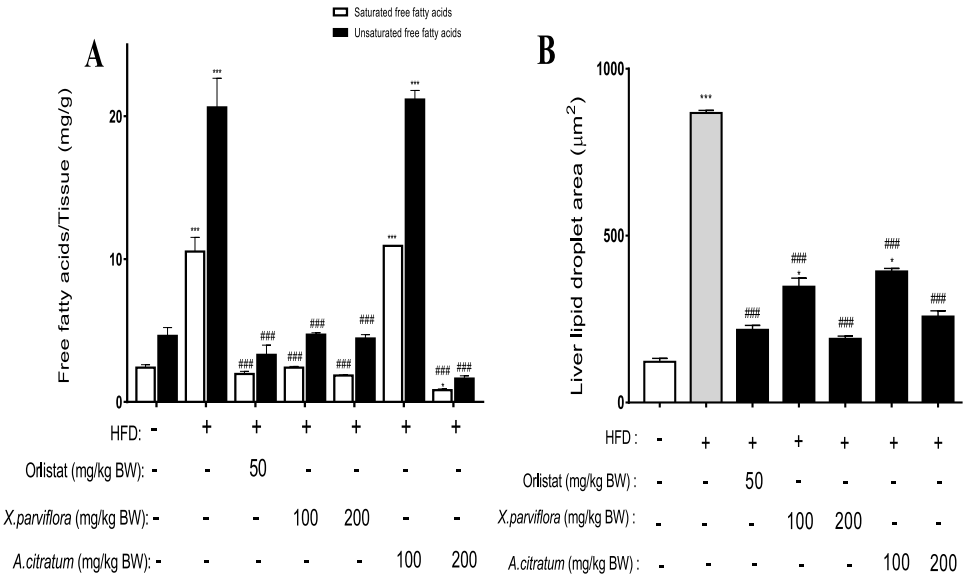


Figure 3. Effect of spice extracts on the liver-saturated/-unsaturated free fatty and lipid droplet formation in HFD-induced obesity in C57BL/6 mice. (A) Effect of spice extracts on saturated and unsaturated fatty acid content in liver tissues of different animal groups after 30 days of treatment. (B) Effect of spice extracts on liver steatosis. The values represent the mean ± SD. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ vs the HFD group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs ND group. HFD: high-fat diet.

quantities were significantly ($p < 0.001$) lowered by the administration of orlistat or 200 mg/kg BW plant extracts.

Effect of Hydroethanolic Spice Extracts on the Liver Lipid Droplet Accumulation. Consistent with the previous findings, significant changes were detected in the number of lipid droplets from liver tissues of all groups stained with hematoxylin and eosin (H&E). The liver section of the mice fed HFD group showed a dramatic ($p < 0.001$) increase in the number of lipids drops (85.89% of lipid droplets) compared to the ND-fed mice group (Figure 3B). These liver sections showed intense hepatic steatosis (microvesicular and macrovesicular lipid droplets). We observed the dilatation of sinusoids, portal triads, centrilobular veins, and the presence of Mallory-Denk bodies with a few or no necroinflammatory foci (Figure 4B). In comparison, only a few lipid droplets were seen in the HFD-fed mice groups and receiving treatment (reduction of 54.78−77.99% lipid droplets) (Figure 3B). Treatment reduced the fat depots in the liver, with a lower presence of microvesicular and macrovesicular lipid droplets, large, round, and fairly euchromatic hepatocytes, absence of Mallory-Denk bodies, and weakly dilated sinusoids and centrilobular veins (Figure 4C−G).

Photomicrographs of histological liver sections of different treatment groups were taken from H&E-stained slides at a magnification of 100×. Microscopic examination and photomicrographs of **A:** normal group shows large, round, and fairly euchromatic nuclei with no inflammatory cells. The black arrow shows a normal centrilobular vein. Black dotted arrows show normal sinusoids. The green arrowhead shows the portal triad. The black arrowhead shows a normal hepatocyte. **B:** HFD-fed group showed severe hepatic steatosis and significantly fewer necroinflammatory foci. Severe fat vacuole accumulation in the cytoplasm of hepatocytes and Mallory-Denk bodies is seen. Sinusoids, the portal triad, and centrilobular veins are dilated. The black arrow shows a dilated centrilobular vein. The black and red arrowheads, respectively, show microvesicular and macrovesicular lipid droplets. The blue arrowhead indicates irregular-shaped eosinophilic aggregates in the cytoplasm of hepatocytes. **C:** HFD-fed + orlistat (50 mg kg^{-1} BW) group, lipid

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

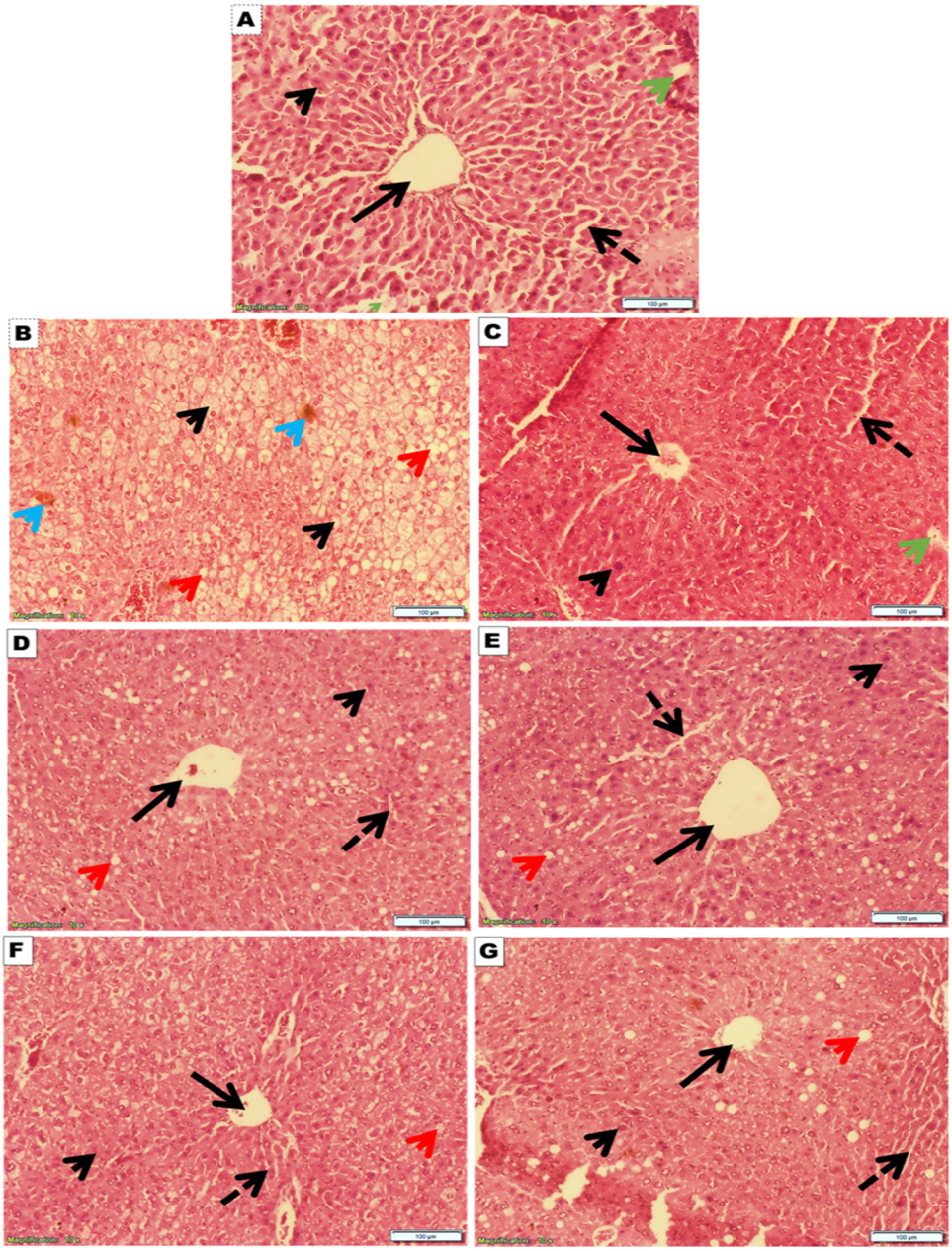


Figure 4. Histological study of liver tissue. Representative microscopic images of liver sections of mice obtained from different treatment groups. The liver sections were stained with H&E, and the magnification was 100. Microscopic examination of (A) Normal group (ND), (B) HFD-fed mice group, (C) HFD-fed + orlistat (50 mg·kg^{-1} BW) (D&E) HFD + XP (**D**:100 mg·kg^{-1} BW and **E**: 200 mg·kg^{-1} BW) and (F&G) AC (**F**:100 mg·kg^{-1} BW and **G**: 200 mg·kg^{-1} BW) treated groups. ND, Normal diet; HFD, High-fat diet; XP, *Xylopia parviflora*; AC, *Aframomum citratum*.

accumulation within the cytoplasm of hepatocytes was considerably decreased compared to the HFD group. The same observations presented in A are shown here. **D&E** and **F&G**: Images from the HFD-fed mice group + *X. parviflora* (**D:**100 mg kg^{-1} BW and **E:** 200 mg kg^{-1} BW) and *A. citratus* (**F:**100 mg kg^{-1} BW and **G:** 200 mg kg^{-1} BW)-treated groups showed signs of fatty change in liver cells, indicating that the extracts prevent the accumulation of triglycerides within hepatocytes, despite the low presence of microvesicular and macrovesicular lipid droplets. Hepatocytes have large, round, and fairly euchromatic nuclei without inflammatory cells. Sinusoids and centrilobular veins are weakly dilated, and Mallory-Denk bodies are absent. The black arrow shows a normal centrilobular vein. Black dotted arrows show normal sinusoids. The red arrowhead shows macrovesicular lipid droplets.

Effect of Hydroethanolic Spice Extracts on Antioxidant Enzymes and Biomarkers of Oxidative Damage. As oxidative stress is known to be one of the factors contributing to the development of fatty liver, for these reasons, we analyzed the oxidative status of the different groups. As shown in Table 3, lipid accumulation significantly ($p < 0.001$) increased for 53.70, 70.66, and 65.44% of TBARS, protein carbonyl, and ROS levels in the liver, respectively, in the HFD-fed mice group compared to the ND-fed mice group. Conversely, orlistat and all the administered doses of spice extracts significantly ($p < 0.001$) reduced the level of oxidative stress biomarkers. However, NO levels did not differ among

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

Table 3. Effect of Spice Extracts on Liver Antioxidant Enzyme Activities and Oxidative Stress Biomarkers in DIO-C57BL/6 Mice Post-Treatment[a]

groups of mice	ND	HFD	HFD + ORL50	HFD + XP100	HFD + XP200	HFD + AC100	HFD + AC200
parameters	Antioxidant enzyme activities and total protein content						
SOD (UI/mg Protein)	1.23 ± 0.15	0.63 ± 0.07***	1.47 ± 0.07* ###	1.09 ± 0.07###	1.11 ± 0.18###	1.08 ± 0.09###	1.2 ± 0.07###
CAT (UI/mg Protein)	0.11 ± 0.17	0.07 ± 0.00*	0.10 ± 0.02	0.06 ± 0.04**	0.17 ± 0.03**##	0.06 ± 2.03**	0.09 ± 0.90
GST (nmol/min/mg Protein)	12.62 ± 3.33	2.93 ± 7.66**	13.24 ± 2.53##	4.01 ± 1.09	11.85 ± 3.57##	9.68 ± 6.39#	13.01 ± 9.01##
protein (mg/mL)	5.03 ± 1.59	5.09 ± 2.022	4.73 ± 0.62	4.52 ± 0.89	4.01 ± 0.21	4.05 ± 0.90	5.18 ± 0.95
	Oxidative stress biomarkers						
LPO (nmol of TBARS/mg Protein)	0.25 ± 0.06	0.54 ± 0.07***	0.36 ± 0.05#	0.29 ± 0.05###	0.29 ± 0.04###	0.26 ± 0.02###	0.23 ± 0.03###
protein carbonyl (nmol/mg Protein)	1.42 ± 0.25	4.84 ± 0.14***	2.71 ± 0.53###	3.61 ± 0.75**	2.59 ± 1.08##	3.44 ± 0.90**	2.99 ± 0.85*##
nitric oxide (nmol/mg Protein)	0.65 ± 0.11	0.59 ± 0.16	0.61 ± 0.07	0.68 ± 0.07	0.68 ± 0.28	0.55 ± 0.04	0.49 ± 0.22
ROS Level (% vs HFD)	34.56 ± 6.36	100 ± 9.71***	44.90 ± 3.10*###	70.07 ± 3.55***###	57.14 ± 3.36***###	45 ± 5.39*###	41.09 ± 2.86###

[a]The values represent the mean ± SD. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ vs the HFD group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs ND group. ND, normal diet; HFDIO, high-fat-diet-induced obesity; HFD, high-fat diet; ORL, orlistat; XP, *Xylopia parviflora*; AC, *Aframomum citratum*; SOD, superoxide dismutase; CAT, catalase GST, glutathion-S-transferase; LPO, lipid peroxidation; TBARS, thiobarbituric acid reactive substance assay; and ROS, reactive oxygen species.

groups. Consistently, enzyme activities of superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST) significantly ($p < 0.001$) decreased by 48.78% ($p < 0.001$), 30% ($p < 0.05$), and 76.78% ($p < 0.01$), respectively, in the HFD-fed mice group when compared with the ND-fed mice group. Oral administration of orlistat and spice extracts at 200 mg/kg BW significantly increased the SOD and GST activities. Nevertheless, only mice receiving *X. parviflora* at 200 mg/kg BW showed a significantly ($p < 0.05$) higher activity of GST.

RP-HPLC and Direct ESI-MS/MS Characterization of Hydroethanolic Spice Extracts. The chemical characterization of the *X. parviflora* and *A. citratum* hydroethanolic extracts analysis was carried out through reverse phase-high performance liquid chromatography-photodiode array detection (RP-HPLC-PDA) and direct electron impact ionization-tandem mass spectrometry (EI-MS/MS) in negative ionization mode. The RP-HPLC-PDA base peak chromatograms were recorded at $\lambda_1 = 280$ nm and $\lambda_2 = 320$ nm (Figures 5 and 1S). Table 4 represents the identified individual compounds, their retention times, and observed molecular and fragmented ions. A total of 10 phenolic compounds have been tentatively identified in the extracts (3 and 7 phenolic compounds in *X. parviflora* and *A. citratum*, respectively) when the MS data of the detected peaks were compared with that reported in the literature and by searching the database of phytochemical dictionary of natural products. The identified compounds of both the extracts belonged to various classes that include mainly phenolic acids and flavonoids (Table 4).

Compounds Identified in *Xylopia parviflora*. Three significant compounds, eluted at different times, were detected through RP-HPLC (peak **1a**, peak **1b**, and peak **1c**). Peak **1a** showed a molecular ion at *m/z* 353.1 and fragmentation ions at *m/z* 191 $[M - H\text{-}162]^-$ as the base peak with the acyl group usually reported to be linked to the 3-OH and *m/z* 179 $[M - H\text{-quinic acid}]^-$. Based on the intensity of the *m/z* 179 fragment (49% intensity), which is higher for the 3-OH isomer compared to that usually detected for the corresponding 5-OH isomer, this compound was identified as 3-O-caffeoylquinic acid.[11] A hydroxycinnamic acid derivative (Peak **1b**) with a molecular ion at *m/z* 325.2 was tentatively identified as p-coumaric acid 4-O-glucoside, producing fragment ions at *m/z* 119 $[M - H - 162 - 44]^-$, thus indicating the loss of a caffeoyl moiety and a carboxylic group.[12] Peak **1c** exhibited maximal absorption at 280 and 320 nm with a molecular ion at *m/z* 335.1. It produced fragments *m/z* 135 $[M - H - \text{quinic acid}-28]^-$ (base peak) with a loss of quinic acid residue and a carboxyl group, as well as ions at *m/z* 179 $[M - H - \text{caffeoyl acid}]^-$ and *m/z* 161 $[M - H - \text{quinic acid}]^-$ with a loss of a caffeoyl moiety and quinic acid residue, respectively. Based on these data and comparing retention times and MS^2 fragments, this compound was assigned as a derivative of shikimic acid, the 5-O-caffeoylshikimic acid.[11,13]

Compounds Identified in *Aframomum citratum*. The chromatographic RP-HPLC and direct ESI-MS/MS profiles of *Aframomum citratum* hydroethanolic extract are shown in supplementary Figure 1S and Table 4, respectively. Seven significant compounds (peak **2a** to peak **2g**), eluted at different times, were detected by comparing with the RP-HPLC chromatogram of water MS-grade used as a control. Compound **2a** revealed a molecular ion at *m/z* 183 and produced fragment ions at *m/z* 168 $[M - H - CH_3]^-$ (base peak), indicating the loss of a methyl radical, *m/z* 139 $[M - H - H_2O]^-$ and *m/z* 139 $[M - H - COO]^-$ indicating the loss of water and carboxylic function. This compound was assigned as 4-*O*-methylgallic acid in agreement with previous reports.[14,15] Peaks **2b** and **2d** showed a molecular ion at *m/z* 419.1 and displayed similar MS^2 fragmentation patterns that mostly produced fractions at *m/z* 197 $[M - H - 162 - 2CH_2O]^-$ and *m/z* 179 $[M - H - 162 - 2CH_2O - H_2O]^-$, indicating the loss of a caffeoyl moiety, methanal group, and water. The abovementioned data showed that the two compounds were from the same series of prenylated flavonoid morusin.[16] Peak **2c** exhibited a molecular ion at *m/z* 325.2 and MS^2 fragmentation patterns similar to those observed for peak **1b**. Thus, comparing the data of both compounds (**1b** and **2c**), peak **2c** was assigned as p-coumaric acid 4-O-glucoside.[13] Peak **2e** showed a molecular ion at *m/z* 433.1, and it produced an abundant fragment at *m/z* 269 $[M - H - 146 - H_2O]^-$ (95% of intensity) corresponding to the loss of moiety of O-linked glucose, and a fragment at *m/z* 287 $[M - H - 146]^-$ indicating the loss of a deoxyhexose moiety. The above fragments were consistent with the known naringenin-7-O-

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

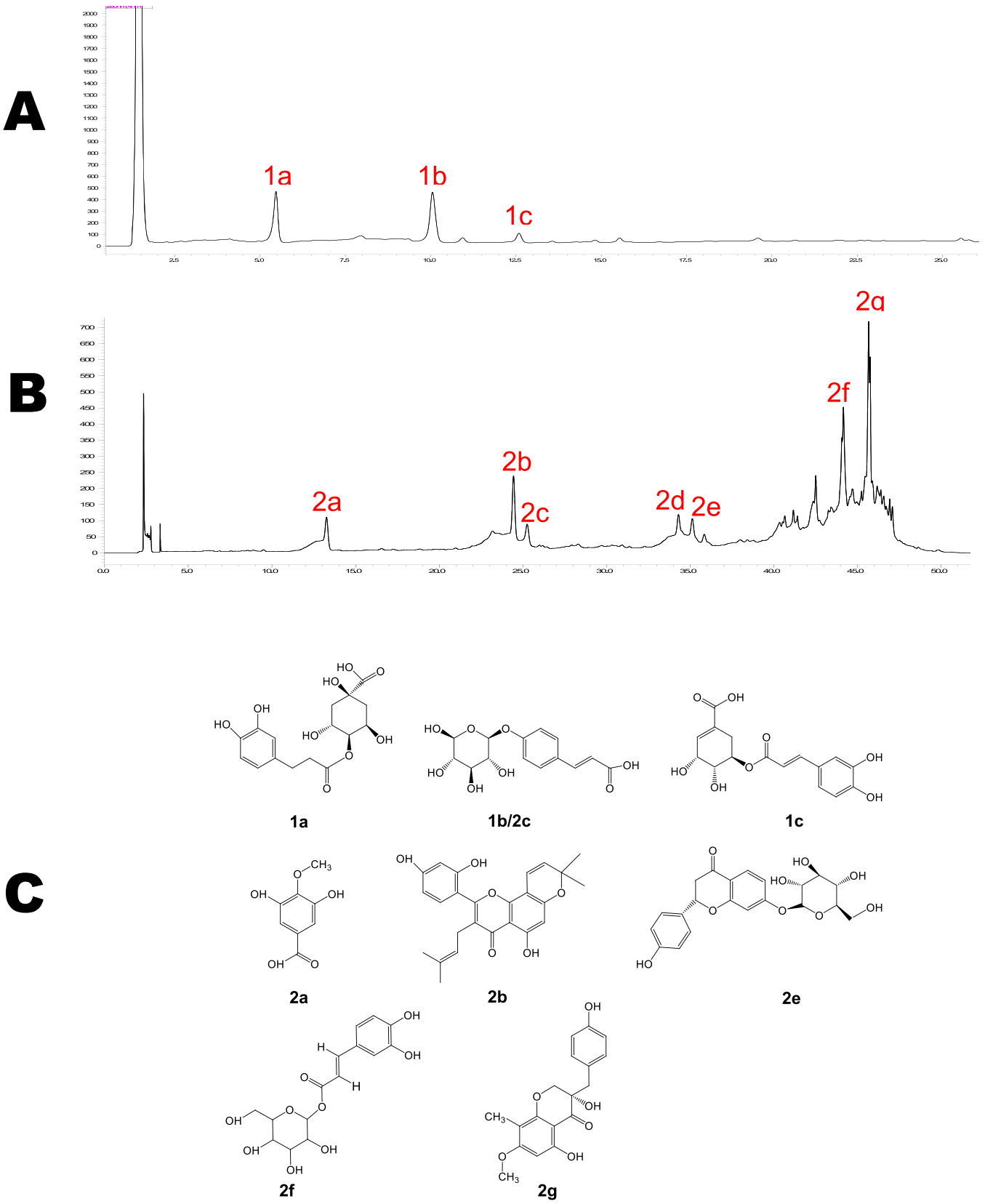


Figure 5. Representative RP-HPLC-PDA chromatogram of spice extracts at 280/320 nm. (A) *Xylopia parviflora*, (B) *Aframomum citratum*. (C) Compounds identified (**1a**: 3-O-caffeoylquinic acid, **1b**/**2c**: p-coumaric acid 4-O-glucoside, **1c**: 5-O-caffeoylshikimic acid, **2a**: 4-*O*-methylgallic acid, **2b**: morusin, **2d**: morusin derivative, **2e**: naringenin-7-O-glucoside, **2f**: caffeic acid-hexose, and **2g**: 5,3,4′-trihydroxy-7-methoxy-8-methyl homoisoflavanone).

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

Table 4. Identification of Polyphenol Compounds in Hydro-Ethanolic Extracts through Direct ESI-MS/MS Analysis

peak N°	RT (min)	Λmax (nm)	precursor ion (m/z)	fragment ions ESI-MS^2 (% base peak)	formula	tentative identification (references)
polyphenol fractions from *Xylopia parviflora* collected through RP-HPLC						
1a	5.46	280, 320	$[M - H]^-$: 353.1	191.0436 (100), 135.0346 (50), 179.0231 (49), 353.0708 (10), 161.0131 (5), 171.0179 (2)	$C_{16}H_{18}O_9$	3-O-caffeoylquinic acid[11]
1b	10.05	280, 320	$[M - H]^-$: 325.2	325.1698 (100), 183.0000 (90), 184.0070 (8), 119.0405 (6), 197.0153 (4), 239.0614 (2)	$C_{15}H_{18}O_8$	*p*-coumaric acid 4-O-glucoside[12]
1c	12.58	280, 320	$[M - H]^-$: 335.1	135.0304 (100), 179.0153 (75), 161.0070 (30), 335.0397 (17), 227.1758 (12), 133.0153 (5)	$C_{16}H_{16}O_8$	5-O-caffeoylshikimic acid[13]
polyphenol fractions from *Aframomum citratum* collected through RP-HPLC						
2a	13.26	280, 320	$[M - H]^-$: 183.0	168.9883 (100), 138.9895 (45), 139.9964 (27), 165.9728 (25), 183.0102 (7), 166.9802 (5)	$C_8H_8O_5$	4-*O*-methyl gallic acid[15]
2b	24.43	280, 320	$[M - H]^-$: 419.1	419.0892 (100), 197.0243 (78), 182.0025 (55), 139.0250 (37), 180.9949 (22), 179.0156 (10)	$C_{25}H_{24}O_6$	morusin[16]
2c	25.25	280, 320	$[M - H]^-$: 325.2	325.1694 (100), 183.0000 (90), 197.0146 (9), 119.0402 (7), 184.0079 (8), 225.0426 (4)	$C_{15}H_{18}O_8$	*p*-coumaric acid 4-O-glucoside[12]
2d	34.29	280, 320	$[M - H]^-$: 419.1	419.1187 (100), 197.0327 (55), 182.0098 (33), 181.0025 (25), 125.0138 (24), 164.0005 (12)	$C_{25}H_{24}O_6$	morusin derivative[16]
2e	35.12	280, 320	$[M - H]^-$: 433.1	151.9999 (100), 269.0310 (95), 433.0966 (70), 259.0471 (40), 287.0404 (38), 286.0331 (14)	$C_{21}H_{22}O_{10}$	naringenin-7-O-glucoside[13]
2f	42.5	280, 320	$[M - H]^-$: 341.1	89.0160 (100), 59.0085 (90), 341.0941 (80), 119.0256 (60), 179.0444 (53), 206.8386 (33)	$C_{15}H_{18}O_9$	caffeic acid hexose[17]
2g	44.12	280, 320	$[M - H]^-$: 329.2	329.1975 (100), 171.0839 (40), 211.1107 (35), 139.0973 (22), 229.1196 (20), 139.0973 (18)		homoisoflavonoid derivative[18]

glucoside and allowed the identification of peak **2e**. Peak **2f**, with a molecular ion at *m/z* 341.1 and fragments at *m/z* 179 $[M - H - 162]^-$, *m/z* 179 $[M - H - 162 - 2CH_2O]^-$ obtained after a loss of caffeoyl moiety, was assigned as caffeic acid hexose.[17] However, it was not possible to identify at which position the caffeoyl group is glycosylated. Peak **2g** displayed the parent ion at *m/z* 329.2 and fragments at *m/z* 139 $[M - H - 162 - CO]^-$, *m/z* at 171 $[M - H - 158]^-$, and *m/z* at 211 $[M - H - 188]^-$. There was no information related to this compound in the literature; however, the observed fragments may refer to a methoxyl group in the A-ring and loss of a methyl radical. According to the fragmentation behavior of the compound **2g**, it could be classified into a homoisoflavonoid group.[18]

DISCUSSION

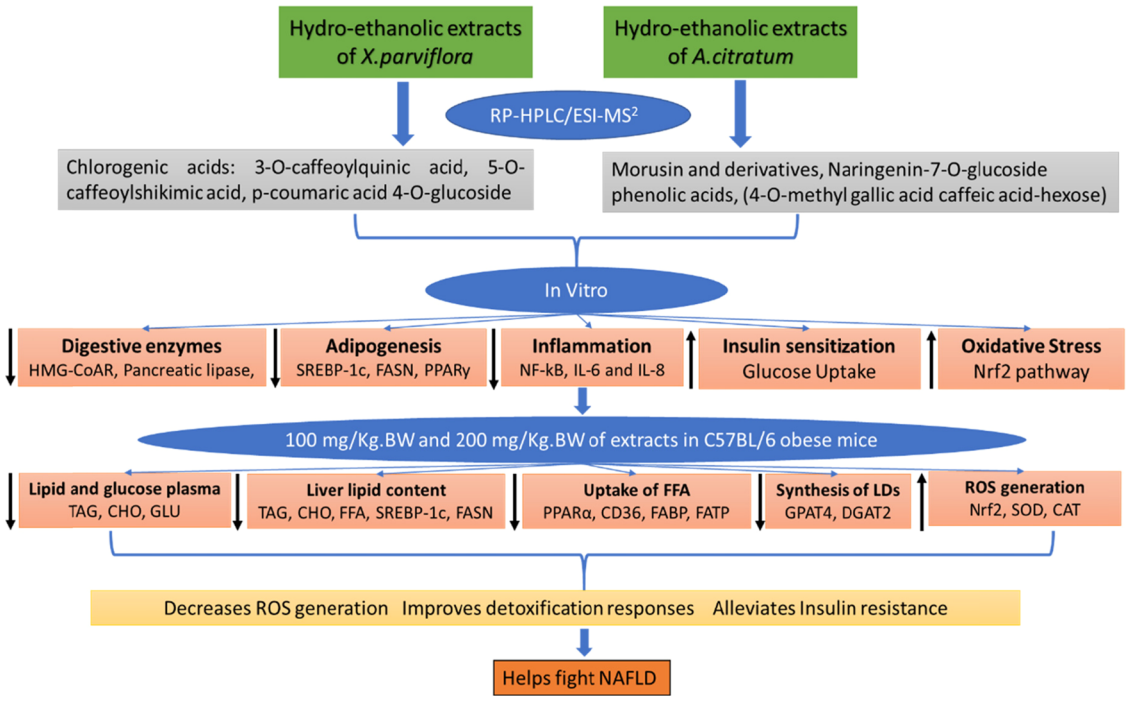
NAFLD is a common chronic liver disease generally associated with high metabolic risk of health problems such as diabetes, obesity, dyslipidemia, insulin resistance, hypertension, atherosclerosis, and hypertriglyceridemia.[19] High calorie intake and unhealthy dietary habits are among the most important risk factors that could lead to the antilipolytic effect of insulin in adipose tissue, increasing FFAs in nonadipose tissues like the liver.[20a] Currently, there is no drug officially approved to treat NAFLD, although there are many drugs in the pipeline.[20b] However, the recommended treatment for NAFLD includes lifestyle modification and some pharmacological interventions classified as lipid-lowering, insulin sensitizers and antioxidant drugs. Herbal medicines have been traditionally used to improve liver conditions.[2] In recent years, there has been a significant advance in the field of drug research, and it has been found that herbal medicines are regarded as rich sources of natural bioactive chemicals that improve hepatic function. In our previous studies,[7−9] several edible plants used as spices in the Cameroon diet showed antidiabetic, antiobesity, anti-inflammatory, and antioxidant activities in cells and cell-free systems. Among those spices were *Xylopia parviflora (A. rich) Benth* and *Aframomum citratum (Pereira ex Oliv. et Hanb.) K. Shum*, which are subject of the present study. We characterized their polyphenol profile and investigated in vivo their beneficial effects on hepatic lipid accumulation. HFD-induced obesity leading to NAFLD and the outcome of treatment with the hydroethanolic spice extracts on gaining weight as well as the lipid profile in plasma and liver tissues in obese *C57BL/6* mice was evaluated.

Several studies have reported that changes in BW were significantly associated with both the development (30−45% weight gain) and remission (7−10% weight loss) of NAFLD.[21,22] In this study, spice extracts and orlistat reduced BW compared with the control HFD group. The BW reduction was significant when obese mice were treated with the extracts at 200 mg/kg BW. Similar results were reported for other botanicals,[19,23] suggesting that spice extracts could probably enhance energy expenditure and fat oxidation, thereby inducing weight loss in obese mice.

The liver is an essential organ and plays an important role in lipid metabolism. An imbalance between lipid deposition and removal leads to lipid accumulation in the liver, which is closely related to increased hepatic lipogenesis, augmented lipid uptake, and decrease in hepatic lipid clearance (FFA oxidation and excretion of very low-density lipoprotein).[23] The mice fed with ND or with HFD and treated with orlistat and spice extracts showed decreased liver weight when compared with the HFD group. The oral administration of spice extracts reduced lipid accumulation in the liver (triglyceride, total cholesterol, and fatty acids) as compared that in the HFD-fed mice. The plasma SGOT, SGPT, alkaline phosphatase, creatinine, and urea levels indicate that there was no damage to the vital organs. Plasma triglycerides, total cholesterol, and HDL cholesterol levels were lowered significantly in the mice treated with spice extracts compared to the HFD-fed mice group. Similar observations were reported by Park et al.[24] Moreover, our previous in vitro studies[7] demonstrated the inhibitory activity of *X. parviflora* and *A. citratum* on enzymes involved in lipid synthesis such as 3-hydroxyl-3-methyl glutaryl coenzyme A (HMG-CoA) reductase. As suggested by Berlanga et al.,[25] the spice extracts may downregulate the expression of downstream SREBP-1c (sterol regulatory element-binding

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

Scheme 1. Proposed Mechanism of the Action of the Hydroethanolic Extracts of *X. parviflora* and *A. citratum*



protein 1c) and FASN (fatty acid synthase) genes, proteins leading to the reduction of lipid accumulation in the liver. In addition, spice extracts and orlistat lowered glucose levels (reduction of 12−21%) in HFD-fed mice. Recent data increasingly support a complex interplay between NAFLD and T2DM. NAFLD has been closely related to insulin resistance and frequently coexists with impaired glucose tolerance (IGT) or type 2 diabetes mellitus.[26] Many studies have reported that *C57BL/6* mice are rendered insulin-resistant because of the increase in the total body fat mass and hepatic lipid accumulation as early as 15 weeks of high-fat feeding.[27,28] In accordance with our previous findings in cell-based studies,[9] the present in vivo study confirms that *X. parviflora* and *A. citratum* positively modulated the uptake of glucose.

The accumulation of FFAs in hepatocytes induces lipid accumulation. This leads to hepatic steatosis and impairs lipid metabolism. Moreover, NAFLD is commonly identified by lipid droplet (LD) abundance in hepatocytes, which contributes to the development and progression of the disease.[29] In the present study, the FFAs (saturated and unsaturated) and lipid droplets were significantly higher in the liver tissues of the HFD-fed mice group, which were reduced by the oral administration of orlistat and the spice extracts. In agreement with the study of Li et al.,[30] we found that in the HFD-fed mice groups, the liver tissues have lower saturated and higher unsaturated fatty acid distribution than mice-fed ND. Furthermore, the spice extracts inhibited the uptake of *n-6* unsaturated FFAs such as linolenic, dihomo-γ-linolenic, and gondoic acids previously reported to be precursors of proinflammatory eicosanoids and involved in the development of NASH.[31] Together, these evidences suggest that treatment with the spice extracts and orlistat may influence the uptake and storage of fatty acid in the liver, at least partly due to altered PPARα activity, fatty acid translocase/cluster of differentiation 36 (CD36), fatty acid transporter proteins (FATP), and caveolins and fatty acid-binding protein (FABP) functions. The expressions of these genes are regulated by peroxisome proliferator-activated receptor alpha (PPARα) transcriptional factor and are reported to be essential for NAFLD development in HFD.[32] In addition, treatment may downregulate the activity of numerous enzymes involved in TAG synthesis, such as acyl-CoA synthetases, acyltransferases glycerol-3-phosphate 4 (GPAT4), and diacylglycerol acyl-transferase 2 (DGAT2), which allow for the expansion of preexisting lipid droplets.[29] Similar results were reported earlier,[33,34] where both have shown that downregulation of lipogenic-gene expression in the liver by *Myristica fragrans Houtt.* and *Morus alba L.* extracts inhibit the progression and development of NAFLD. The excess of FFAs can disturb mitochondrial metabolism and increase ROS generation.[22] Also, with the progression of NAFLD toward NASH, the hepatocytes lose their ability to respond to injury as they become increasingly sensitive to damage caused due to the accumulation of toxic lipid metabolites, production of ROS, and dysfunction of detoxification responses.[35] Lipid accumulation in the liver due to HFD induces oxidative stress in mice. This is demonstrated by the high hepatic level of TBARS, protein carbonyl, ROS, and low enzyme activities of SOD, catalase, and GST in the HFD-fed mice group compared with the ND-fed mice group. Spice extracts and orlistat treatments reverted the increase in the above parameters involved in oxidative stress. These data suggest that the antioxidant effect of spice extracts might be beneficial against hepatic fat accumulation. Indeed, various signaling pathways have been described to explain the therapeutic effects of antioxidants, such as the activation of the nuclear factor E2-related factor 2 (Nrf2) pathway and attenuation of nuclear factor-kappa B (NF-κB) signaling.[36] These mechanisms that were already investigated in our previous studies[8,9] in cell models could justify the antioxidant activity of the spice extracts observed in

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

the liver and consequently the inhibition of NAFLD development.

Consistent with the previous findings, two groups of polyphenols (phenolic acids and flavonoids) were detected in the spice extracts. Stereoisomers of chlorogenic acids (3-O-caffeoylquinic acid and 5-O-caffeoylshikimic acid) and hydroxycinnamic acid derivatives (p-coumaric acid 4-O-glucoside) were explicitly identified as the most abundant polyphenols in *X. parviflora* hydroethanolic spice extracts. This is also in accordance with our previous findings.[7] A wide range of experimental studies has confirmed the efficacy of phenolic acids against hepatic steatosis, insulin resistance, and oxidative stress in mice with HFD-induced NAFLD, independent of other lifestyle factors.[19,37] Therefore, this could potentially explain the beneficial effect of *X. parviflora* on hepatic lipid accumulation. Furthermore, flavonoids such as morusin and derivatives, naringenin-7-O-glucoside, and phenolic acids (4-*O*-methyl gallic acid and caffeic acid hexose) were identified in *A. citratum* that might be beneficial against the progression of NAFLD. However, no data or scientific work have clearly demonstrated the role of morusin or morusin derivatives in NALFD. Some experimental evidence from in vivo and in vitro models suggests that flavonoids from dietary plants exhibit hepatoprotective effects, including the improvement of metabolic function, impairment of lipogenesis, reduction of inflammation, and improving liver histology,[38] thus suggesting that the synergic action of *A. citratum*-rich polyphenols could justify the therapeutic effect observed in obesity-induced NAFLD mice. The summary of the findings is schematically shown in Scheme 1.

Our study demonstrated that the oral administration of hydroethanolic *Xylopia parviflora (A. Rich.)* Benth and *Aframomum citratum (Pereira ex Oliv. et Hanb.) K. Shum* spice extracts decreased hepatic lipid accumulation in HFD-induced obese *C57BL/6* mice. Reduction of BW, alleviation of plasma glucose and lipid parameters, improvement of antioxidant status, and hepatoprotective activity against steatosis mediated by phenolic compounds are among the main potential mechanisms of action of these spice extracts. However, the deduction of molecular mechanisms underlying these effects will require further investigations. These plants are well-known, safe, widely distributed in eastern and central Africa. The results, although promising, cannot be directly translated onto the human context but will serve as a lead to further studies in this direction toward treatment against NAFLD.

MATERIALS AND METHODS

Chemicals and Reagents. Biochemical reagents were supplied from Agape Pharmaceutical (Kerala, India). Casein was purchased from Indian Casein Company (Mumbai, India). AIN 93Vx vitamin mixture and AIN 93 M mineral mixture were purchased from MP Biomedicals Pvt. Ltd. (Mumbai, India). Sucrose and lard were purchased from a local market, and other dietary materials were purchased from Himedia (Mumbai, India). The solvents acetonitrile, methanol, isopropanol, and hexane of HPLC grade were purchased from Spectrochem Pvt. Ltd., (Mumbai, India). MS-grade water, methanol, and acetonitrile were purchased from Sigma-Aldrich (Bangalore, India). Ultrapure triple-distilled and MilliQ water prepared using the Millipore (Merck, Mumbai, India) water purification system was used to prepare reagents and buffers throughout the experiment to avoid any metal contamination. All other chemicals and solvents used in the study were procured from Sigma Chemicals Co. (St. Louis, MO, USA) or Merck Pvt. Ltd., (Mumbai, India).

Plant Materials and Sample Preparation. *Xylopia parviflora (A. Rich.) Benth* seeds and *Aframomum citratum (Pereira ex Oliv. Et Hanb.)* fruits were harvested in various localities of the region of West Cameroon, as previously described by Nwakiban et al.[7,9] One hundred grams of specific plant materials were cleaned and stored at room temperature (30 ± 6 °C). Then, the plant materials were powdered and extracted by stirring with 100 mL of ethanol and water (hydroalcoholic) in the ratio 70:30 each, as described earlier.[8] The lyophilized extracts were maintained at 4 °C, and 10 mg was freshly dissolved in 1 mL of HPLC grade or MS grade methanol for chemical characterization of polyphenols. On the other hand, an equal ratio (1:1) of the hydroethanolic solvent was used to dissolve the extracts intended for animal studies.

High-Performance Liquid Chromatography and Electrospray Ionization Mass Spectrometry (ESI-MS/MS) of Phenolic Fractions. *HPLC Analysis.* The phenolic compounds present in each spice extract were analyzed, as previously described[39] by HPLC (Nexera X-2 LC-30A, Shimadzu, Japan). Chromatographic separations were performed on a Chromasol RP-C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase was composed of water adjusted to a pH of 2.65 with acetic acid (solvent A) and solvent B formed with 20% solvent A and 80% acetonitrile. An aliquot of 30 μL of sample solution was injected into the HPLC system, and a linear elution gradient was applied as follows: 0−20% B in 0−35 min, 20−50% B in 35−40 min, 50−100% B in 40−45 min, and 100−0% B in 45−60 min. The column temperature was maintained at 20 °C. The flow rate was 1.2 mL/min, and detection was carried out using a PDA detector, with λ_1 = 280 and λ_1 = 320 nm as preferred wavelengths with the aid of LabSolutions software (version 6.50, Shimadzu, Japan). The peaks generated by the substances of interest were then collected in 1 mL vials for electrospray ionization mass spectrometry (ESI-MS/MS) analysis in off-line mode.

Mass Spectrometry Analysis by ESI-MS/MS. The HPLC fractions were diluted using MS-grade methanol and directly injected to the ESI source through a syringe pump at 0.4 mL/min flow rate. ESI-MS analyses were carried out in the negative ion mode within the *m/z* range 100−2000 *m/z* with an IRDx resolution of 15,000, using a Hybrid Quadrupole-TOF LC/MS/MS mass spectrometer (SCIEX Triple TOF 5600, Singapore). The typical ESI conditions were as follows: gases were GS1−45, GS2−60, and Curtain GAS (CUR)-40, source voltage was 4.0 kV, duospray ion source set at 400 °C, and the collision energy was 10 V. CID-MS/MS experiments were performed on mass-selected precursor ions using standard isolation and excitation configuration. All data acquisition and analysis were performed using the Peak View 2.1 Software (AB SCIEX Triple TOF 5600, Singapore), equipped with MasterView (Version 1.0, AB SCIEX). The XIC manager tool in Master View was used to detect quasimolecular weights, mass errors, and isotope patterns of both targeted and nontargeted compounds. ChemSpider, mzCloud and Chemdraw software, elemental composition analysis, as well as literature review were used to define consistent tentative structures for the identified compounds.

Animal Models and Experimental Design. Male 4−6-week-old *C57BL/6* mice (56) weighing an average of 23 ± 2 g were obtained from CFTRI animal facility after a mandatory

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

institutional clearance with approval number CFT/IAEC/197/2020. The procedures were performed according to India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations usage of laboratory animals, and care was taken to minimize the suffering of animals utilized in this study (approval number: CFT/IAEC/197/2020). Animals were accommodated under standard conditions of temperature 22−25 °C, 12 h light/dark cycle, and relative humidity of 50 ± 10%, with *ad libitum* food (standard chow) and water. After 6 days of acclimatization, mice were randomly separated into seven groups (n = 8 animals per group). One group received a routine diet (10% Kcal fat content) as standard (ND), and other groups were fed with an HFD (60% Kcal by fat) for 18 weeks. Among groups fed with an HFD, only animals showing an increase in weight >30% were selected and divided into six (06) groups (n = 7 animals per group). Before treatment, mice were acclimatized for 2 days, fasted for 16 h, and blood was collected through the retro-orbital plexus.

Randomization of groups was done based on BW, and the experimental design was as follows: mice fed a regular diet with saline through oral gavage (ND + saline); mice fed an HFD with saline through oral gavage (HFD + saline); mice fed an HFD with orlistat through the oral gavage (50 mg/kg BW, HFD + ORL50); mice fed an HFD with *X. parviflora* extract through the oral gavage (100 mg/kg BW, HFD + XP100 and 200 mg/kg BW, HFD + XP200); and mice fed an HFD with *A. citratum* extract through the oral gavage (100 mg/kg BW, HFD + AC100 and 200 mg/kg BW, HFD + AC200). Orlistat and both XP and AC were prepared in saline. The oral treatment was administered every 24 h for 30 days. BWs and FI were recorded every day, and at the end of the experiment, blood was collected through the retro-orbital plexus under anesthesia. The animals were then sacrificed, and the organs were removed, cleaned, weighed, and stored at −80 °C for further studies.

Biochemical Plasma Analysis. Plasma was separated from the blood of mice (n = 7 animals per group) by centrifugation at 2000 rpm for 15 min at 4 °C and immediately transferred into fresh tubes. Lipid parameters such as triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and other biochemical parameters like glucose, protein, creatinine, and urea were estimated in the plasma using commercially available kits (Agape Pharmaceutical, Kerala, India). Plasma enzymes SGPT, SGOT, and ALP were assayed to ascertain that the treatment has not affected any vital functions.

Lipidomic Profile of the Liver Tissue. Total lipids were isolated from the liver tissue of representative mice (n = 5 animals per group) using the Folch method.[40] Total triglycerides and TC content were estimated using colorimetric methods described by Fletcher,[41] Searcy, and Bergquis.[42] The FFA profile (FAMEs: fatty acid methyl esters) of total lipids extracted from liver tissues was determined through GC−MS, as described by Prasad et al.[43] and slightly modified by Dalmia and Tumaney.[44] FAMEs were prepared by the esterification of fat extracted from the liver with 1 mL of BF3-methanol complex solution at 65 °C for 30 min. Fifty microliters of internal standard heptadecanoic acid (1 mg/mL) were added to the samples before methylation. The reaction was allowed to cool for 10 min. After cooling, water: hexane (1:1 v/v) was added to the mixture, and the upper layer that contains FAMEs was transferred to GC vials. The resulting FAMEs were passed through an Agilent DB-23 column ((50%-cyanopropyl)-methylpolysiloxane; 60 m length; 0.25 mm ID; 0.25 μm film thickness) on the Agilent 7890B GC-5977A MSD system (M/S Agilent Technologies, Singapore). One microliter of the sample was injected, and the split ratio was 20:1. Helium at a flow rate of 1 mL/min was used as a carrier gas. The temperature was programmed as follows: the initial temperature was 40 °C for 1 min and increased to 130 °C at a rate of 70 °C/min with a 1 min hold, then ramped up to 170 °C at 6.5 °C/min rates and further increased to 200 °C at 2.75 °C/min rates with 6 min hold. Finally, the temperature was increased to 210 °C at 40 °C/min rate with a 2 min hold, and then increased to 230 °C at 20 °C/min with 7 min hold time. The mass spectrum was recorded under EI (electron impact ionization) at 70 eV (fixed electron energy). The mass range of m/z was between 40−500 Da, and the identified fatty acids were confirmed using mass spectral library search (NIST version 2.0 g).

Hepatic Antioxidant Enzyme Activity and Oxidative Stress Biomarker Analysis. One hundred milligrams of fresh liver tissue samples from representative mice were collected and homogenized in 1 mL of ice-cold sucrose (0.32 M) solution. Liver homogenate samples (n = 5 animals per group) were centrifuged at 6000 rpm for 10 min at 4 °C, and supernatants were used to determine SOD,[45] catalase (CAT),[46] GST activity.[47] The liver protein concentration was quantified using Lowry's method.[48] The oxidative stress biomarkers level viz., reactive oxygen species (ROS),[49] lipid peroxidation (LPO) through thiobarbituric acid reactive substance (TBARS) assay,[50] protein carbonyl (PC),[51] and nitric oxide (NO)[52] concentrations were assayed.

Histological Analyses of Liver Tissues. Sections of the liver tissue from representative mice (n = 3 animals per group) in each group were fixed in 10% formalin, and thin sections of 4−5 μm were mounted on glass slides. The liver sections were stained with H&E, and digital images at ×10 magnification were obtained using a high-resolution camera-mounted optical microscope (BX51, Olympus, Tokyo) connected to a computer. FIJI software was used to measure the area of lipid droplets in order to detect hepatic steatosis.

Statistical Analysis. All results are expressed as mean (±SD). Statistical data were determined with one-way analysis of variance (ANOVA) followed by multiple comparison analysis performed with the Bonferroni post hoc test. To analyze BW, FI, and fatty acid profile, two-way ANOVA followed by multiple comparison analysis performed with a post hoc Tukey test was used. Statistical analyses were calculated, and graphs were prepared using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00050.

Figure 1S. MS^2 spectra of fractions from spice extracts collected through RP-HPLC; Table 1S. Effect of spice extracts on vital organ weights expressed as a BW ratio; Table 2S. Effect of spice extracts on plasma biochemical parameters in HFDIO C57BL/6 mice post and pretreatment. (PDF)

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

AUTHOR INFORMATION

Corresponding Author

Uma Venkateswaran Manjappara − *Department of Lipid Science, CSIR-Central Food Technological Research Institute (CFTRI), Mysore 570 020, India;* orcid.org/0000-0002-3209-1205; Email: umamanjappara@cftri.res.in

Authors

Achille Parfait Nwakiban Atchan − *Department of Biochemistry, Faculty of Science, University of Dschang, Dschang 67, Cameroon;* orcid.org/0000-0003-1660-7125

Shilpa Talkad Shivashankara − *Department of Lipid Science, CSIR-Central Food Technological Research Institute (CFTRI), Mysore 570 020, India*

Stefano Piazza − *Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan 20133, Italy*

Armelle Deutou Tchamgoue − *Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, Yaoundé 13033, Cameroon*

Giangiacomo Beretta − *Department of Environmental Science and Policy, Università degli Studi di Milano, Milan 20133, Italy*

Mario Dell'Agli − *Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan 20133, Italy*

Paolo Magni − *Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan 20133, Italy; IRCCS MultiMedica, Sesto San Giovanni, Milan 20099, Italy*

Gabriel Agbor Agbor − *Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, Yaoundé 13033, Cameroon*

Jules-Roger Kuiaté − *Department of Biochemistry, Faculty of Science, University of Dschang, Dschang 67, Cameroon*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c00050

Author Contributions

Conceptualization: A.P.N.A., G.A.A., G.B., M.D.A., P.M., J.-R.K., and U.V.M.; Writing-original draft: A.P.N.A.; Writing-review: A.P.N.A., S.T.S., G.B., M.D.A., P.M., J.-R.K., and U.V.M.; Methodology: A.P.N.A. and S.T.S.; Software: A.P.N.A.; Formal analysis: A.P.N.A. and S.T.S; Data curation: A.P.N.A. and S.T.S.; Investigators: A.D.T. and S.P.; Resources: A.D.T. and S.P.; Editing: G.A.A., G.B., M.D.A., P.M., and U.V.M.; Supervision: G.A.A., J.-R.K., and U.V.M.; Project administration: U.V.M.

Funding

This work was supported by The World Academy of Science (TWAS) in Trieste, Italy, and the Department of Biotechnology (DBT) in New Delhi, India (Grant No. 3240300014). CSIR-Central Food Technological Research Institute, Mysore, India, Grant No. MLP-263.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Counsil of Scientific Industrial Research-Central Food Technological Research Institute (CSIR-CFTRI), especially the department of Lipid Science (LS), for support with analyses and the use of equipment.

ABBREVIATIONS

AC	*Aframomum citratum*
ALP	alkaline phosphatase
BW	body weight
CAT	catalase
CFTRI	Central Food Technological Research Institute
DBT	Department of Biotechnology
DGAT2	diacylglycerol acyltransferase 2
ESI	eectrospray ionization
FAMEs	fatty acid methyl esters
FFA	free fatty acid
FASN	fatty acid synthase
GC	gas chromatography−mass spectrometry
GST	glutathione S-transferase
HDL	low high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol HFD: high-fat diet
HMG-CoA	3-hydroxyl-3-methyl glutaryl coenzyme
HPLC	high pressure liquid chromatography
IGT	glucose tolerance
LD	lipid droplet
LPO	lipid peroxidation
MetS	metabolic syndrome
MS	mass spectrometry
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
ND	normal diet
NF-κB	nuclear factor-kappa B
NO	nitric oxide
Nrf2	nuclear factor E2-related factor 2
ORL	Orlistat
PC	protein carbonyl
ROS	reactive oxygen species
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SOD	superoxide dismutase
SREBP-1c	sterol regulatory element-binding protein 1c
TAG	triacylglycerol
TBARS	thiobarbituric acid reactive substance
TC	total cholesterol
TG	triglyceride
TWAS	The World Academy of Science
XP	*Xylopia parviflora*

REFERENCES

(1) Setroame, A. M.; Kormla Affrim, P.; Abaka-Yawson, A.; Kwadzokpui, P. K.; Eyram Adrah, F.; Bless, H.; Mohammed, L.; Bawah, A. T.; Alidu, H. W. Prevalence of Metabolic Syndrome and Nonalcoholic Fatty Liver Disease among Premenopausal and Postmenopausal Women in Ho Municipality: A Cross-Sectional Study. *Biomed Res. Int.* **2020**, *2020*, No. e2168381.

(2) Xu, Y.; Guo, W.; Zhang, C.; Chen, F.; Tan, H. Y.; Li, S.; Wang, N.; Feng, Y. Herbal Medicine in the Treatment of Non-Alcoholic Fatty Liver Diseases-Efficacy, Action Mechanism, and Clinical Application. *Front. Pharmacol.* **2020**, *11*, 601.

(3) Raza, S.; Rajak, S.; Anjum, B.; Sinha, R. A. Molecular Links between Non-Alcoholic Fatty Liver Disease and Hepatocellular Carcinoma. *Hepatoma Res.* **2019**, *5*, 42.
(4) Godoy-Matos, A. F.; Silva Júnior, W. S.; Valerio, C. M. NAFLD as a Continuum: From Obesity to Metabolic Syndrome and Diabetes. *Diabetol. Metab. Syndr.* **2020**, *12*, 60.
(5) Schuppan, D.; Schattenberg, J. M. Non-Alcoholic Steatohepatitis: Pathogenesis and Novel Therapeutic Approaches. *J. Gastroenterol. Hepatol.* **2013**, *28*, 68−76.
(6) Kasper, P.; Martin, A.; Lang, S.; Kütting, F.; Goeser, T.; Demir, M.; Steffen, H.-M. NAFLD and Cardiovascular Diseases: A Clinical Review. *Clin. Res. Cardiol.* **2021**, *110*, 921−937.
(7) Atchan Nwakiban, A. P.; Sokeng, A. J.; Dell'Agli, M.; Bossi, L.; Beretta, G.; Gelmini, F.; Deutou Tchamgoue, A.; Agbor Agbor, G.; Kuiaté, J.-R.; Daglia, M.; Magni, P. Hydroethanolic Plant Extracts from Cameroon Positively Modulate Enzymes Relevant to Carbohydrate/Lipid Digestion and Cardio-Metabolic Diseases. *Food Funct.* **2019**, *10*, 6533−6542.
(8) Atchan Nwakiban, A. P.; Cicolari, S.; Piazza, S.; Gelmini, F.; Sangiovanni, E.; Martinelli, G.; Bossi, L.; Carpentier-Maguire, E.; Deutou Tchamgoue, A.; Agbor, G.; Kuiaté, J. R.; Beretta, G.; Dell'Agli, M.; Magni, P. Oxidative Stress Modulation by Cameroonian Spice Extracts in HepG2 Cells: Involvement of Nrf2 and Improvement of Glucose Uptake. *Metabolites* **2020**, *10*, 182.
(9) Nwakiban, A. P. A.; Fumagalli, M.; Piazza, S.; Magnavacca, A.; Martinelli, G.; Beretta, G.; Magni, P.; Tchamgoue, A. D.; Agbor, G. A.; Kuiaté, J.-R.; Dell'Agli, M.; Sangiovanni, E. Dietary Cameroonian Plants Exhibit Anti-Inflammatory Activity in Human Gastric Epithelial Cells. *Nutrients* **2020**, *12*, 3787.
(10) Atchan Nwakiban, A. P.; Passarelli, A.; Da Dalt, L.; Olivieri, C.; Demirci, T. N.; Piazza, S.; Sangiovanni, E.; Carpentier-Maguire, E.; Martinelli, G.; Shivashankara, S. T.; Manjappara, U. V.; Tchamgoue, A. D.; Agbor, G. A.; Kuiate, J.-R.; Daglia, M.; Dell'Agli, M.; Magni, P. Cameroonian Spice Extracts Modulate Molecular Mechanisms Relevant to Cardiometabolic Diseases in SW 872 Human Liposarcoma Cells. *Nutrients* **2021**, *13*, 4271.
(11) Carazzone, C.; Mascherpa, D.; Gazzani, G.; Papetti, A. Identification of Phenolic Constituents in Red Chicory Salads (Cichorium Intybus) by High-Performance Liquid Chromatography with Diode Array Detection and Electrospray Ionisation Tandem Mass Spectrometry. *Food Chem.* **2013**, *138*, 1062−1071.
(12) Ibrahim, R. M.; el-Halawany, A. M.; Saleh, D. O.; El Naggar, E. M. B.; el-Shabrawy, A. E. R. O.; el-Hawary, S. S. HPLC-DAD-MS/MS Profiling of Phenolics from Securigera Securidaca Flowers and Its Anti-Hyperglycemic and Anti-Hyperlipidemic Activities. *Rev. Bras. Farmacogn.* **2015**, *25*, 134−141.
(13) Kang, J.; Price, W. E.; Ashton, J.; Tapsell, L. C.; Johnson, S. Identification and Characterization of Phenolic Compounds in Hydromethanolic Extracts of Sorghum Wholegrains by LC-ESI-MS(n). *Food Chem.* **2016**, *211*, 215−226.
(14) Li, C.; Seeram, N. P. Ultra-Fast Liquid Chromatography Coupled with Electrospray Ionization/Time-of- Flight Mass Spectrometry for Rapid Phenolic Profiling of Red Maple (Acer Rubrum) Leaves. *J. Sep. Sci.* **2018**, *41*, 2331−2346.
(15) Tang, J.; Dunshea, F. R.; Suleria, H. A. R. LC-ESI-QTOF/MS Characterization of Phenolic Compounds from Medicinal Plants (Hops and Juniper Berries) and Their Antioxidant Activity. *Foods* **2020**, *9*, 7.
(16) Gómez, J.; Simirgiotis, M. J.; Manrique, S.; Lima, B.; Bórquez, J.; Feresin, G. E.; Tapia, A. UHPLC-HESI-OT-MS-MS Biomolecules Profiling, Antioxidant and Antibacterial Activity of the "Orange-Yellow Resin" from Zuccagnia Punctata Cav. *Antioxidants* **2020**, *9*, 123.
(17) Gouveia, S.; Castilho, P. C. Characterisation of Phenolic Acid Derivatives and Flavonoids from Different Morphological Parts of Helichrysum Obconicum by a RP-HPLC-DAD-(−)-ESI-MS n Method. *Food Chem.* **2011**, *129*, 333−344.
(18) Ye, M.; Guo, D.; Ye, G.; Huang, C. Analysis of Homoisoflavonoids in Ophiopogon Japonicus by HPLC-DAD-ESI-MSn. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 234−243.
(19) Davoodi, I.; Rahimi, R.; Abdollahi, M.; Farzaei, F.; Farzaei, M. H.; Memariani, Z.; Najafi, F. Promising Effect of Rosa Damascena Extract on High-Fat Diet-Induced Nonalcoholic Fatty Liver. *J. Tradit. Complement. Med.* **2017**, *7*, 508−514.
(20) (a) Bertot, L. C.; Adams, L. A. Trends in Hepatocellular Carcinoma Due to Non-Alcoholic Fatty Liver Disease. *Expert Rev. Gastroenterol. Hepatol.* **2019**, *13*, 179−187. (b) Negi, C. K.; Babica, P.; Bajard, L.; Bienertova-Vasku, J.; Tarantino, G. Insights into the molecular targets and emerging pharmacotherapeutic interventions for nonalcoholic fatty liver disease. *Metabolism* **2022**, *126*, No. 154925.
(21) Brunner, K. T.; Henneberg, C. J.; Wilechansky, R. M.; Long, M. T. Nonalcoholic Fatty Liver Disease and Obesity Treatment. *Curr. Obes. Rep.* **2019**, *8*, 220−228.
(22) Yoshioka, N.; Ishigami, M.; Watanabe, Y.; Sumi, H.; Doisaki, M.; Yamaguchi, T.; Ito, T.; Ishizu, Y.; Kuzuya, T.; Honda, T.; Ishikawa, T.; Haruta, J.; Fujishiro, M. Effect of Weight Change and Lifestyle Modifications on the Development or Remission of Nonalcoholic Fatty Liver Disease: Sex-Specific Analysis. *Sci. Rep.* **2020**, *10*, 481.
(23) Wang, L.-F.; Wang, X.-N.; Huang, C.-C.; Hu, L.; Xiao, Y.-F.; Guan, X.-H.; Qian, Y.-S.; Deng, K.-Y.; Xin, H.-B. Inhibition of NAMPT Aggravates High Fat Diet-Induced Hepatic Steatosis in Mice through Regulating Sirt1/AMPKα/SREBP1 Signaling Pathway. *Lipids Health Dis.* **2017**, *16*, 82.
(24) Park, J. U.; Kang, J. H.; Rahman, M. A. A.; Hussain, A.; Cho, J. S.; Lee, Y. I. Gastroprotective Effects of Plants Extracts on Gastric Mucosal Injury in Experimental Sprague-Dawley Rats. *BioMed Res. Int.* **2019**, *2019*, No. 8759708.
(25) Berlanga, A.; Guiu-Jurado, E.; Porras, J. A.; Auguet, T. Molecular Pathways in Non-Alcoholic Fatty Liver Disease. *Clin. Exp. Gastroenterol.* **2014**, *7*, 221−239.
(26) Williams, K. H.; Shackel, N. A.; Gorrell, M. D.; McLennan, S. V.; Twigg, S. M. Diabetes and Nonalcoholic Fatty Liver Disease: A Pathogenic Duo. *Endocr. Rev.* **2013**, *34*, 84−129.
(27) Lo, L.; McLennan, S. V.; Williams, P. F.; Bonner, J.; Chowdhury, S.; McCaughan, G. W.; Gorrell, M. D.; Yue, D. K.; Twigg, S. M. Diabetes Is a Progression Factor for Hepatic Fibrosis in a High Fat Fed Mouse Obesity Model of Non-Alcoholic Steatohepatitis. *J. Hepatol.* **2011**, *55*, 435−444.
(28) Perry, R. J.; Samuel, V. T.; Petersen, K. F.; Shulman, G. I. The Role of Hepatic Lipids in Hepatic Insulin Resistance and Type 2 Diabetes. *Nature* **2014**, *510*, 84−91.
(29) Mashek, D. G. Hepatic Lipid Droplets: A Balancing Act between Energy Storage and Metabolic Dysfunction in NAFLD. *Mol. Metab.* **2021**, *50*, No. 101115.
(30) Li, M.; Fu, W.; Li, X.-A. Differential Fatty Acid Profile in Adipose and Non-Adipose Tissues in Obese Mice. *Int. J. Clin. Exp. Med.* **2010**, *3*, 303−307.
(31) Kawaguchi, T.; Itou, M.; Taniguchi, E.; Sata, M. Exendin-4, a Glucagon-like Peptide-1 Receptor Agonist, Modulates Hepatic Fatty Acid Composition and Δ-5-desaturase Index in a Murine Model of Non-alcoholic Steatohepatitis. *Int. J. Mol. Med.* **2014**, *34*, 782−787.
(32) Ampong, I.; Watkins, A.; Gutierrez-Merino, J.; Ikwuobe, J.; Griffiths, H. R. Dietary Protein Insufficiency: An Important Consideration in Fatty Liver Disease? *Brit. J. Nutr.* **2020**, *123*, 601−609.
(33) Wu, C.; Luan, H.; Wang, S.; Zhang, X.; Wang, R.; Jin, L.; Guo, P.; Chen, X. Modulation of Lipogenesis and Glucose Consumption in HepG2 Cells and C2C12 Myotubes by Sophoricoside. *Molecules* **2013**, *18*, 15624−15635.
(34) Zhao, W.; Song, F.; Hu, D.; Chen, H.; Zhai, Q.; Lu, W.; Zhao, J.; Zhang, H.; Chen, W.; Gu, Z.; Wang, G. The Protective Effect of Myristica Fragrans Houtt. Extracts Against Obesity and Inflammation by Regulating Free Fatty Acids Metabolism in Nonalcoholic Fatty Liver Disease. *Nutrients* **2020**, *12*, 2507.

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928

(35) Overi, D.; Carpino, G.; Franchitto, A.; Onori, P.; Gaudio, E. Hepatocyte Injury and Hepatic Stem Cell Niche in the Progression of Non-Alcoholic Steatohepatitis. *Cell* **2020**, *9*, 590.
(36) Li, S.; Tan, H. Y.; Wang, N.; Cheung, F.; Hong, M.; Feng, Y. The Potential and Action Mechanism of Polyphenols in the Treatment of Liver Diseases. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 1−25.
(37) Salomone, F.; Ivancovsky-Wajcman, D.; Fliss-Isakov, N.; Webb, M.; Grosso, G.; Godos, J.; Galvano, F.; Shibolet, O.; Kariv, R.; Zelber-Sagi, S. Higher Phenolic Acid Intake Independently Associates with Lower Prevalence of Insulin Resistance and Non-Alcoholic Fatty Liver Disease. *JHEP Rep.* **2020**, *2*, No. 100069.
(38) Yao, H.; Qiao, Y.-J.; Zhao, Y.-L.; Tao, X.-F.; Xu, L.-N.; Yin, L.-H.; Qi, Y.; Peng, J.-Y. Herbal Medicines and Nonalcoholic Fatty Liver Disease. *World J. Gastroenterol.* **2016**, *22*, 6890−6905.
(39) Daga, P.; Vaishnav, S. R.; Dalmia, A.; Tumaney, A. W. Extraction, Fatty Acid Profile, Phytochemical Composition and Antioxidant Activities of Fixed Oils from Spices Belonging to Apiaceae and Lamiaceae Family. *J. Food Sci. Technol.* **2022**, *59*, 518−531.
(40) Folch, J.; Lees, M.; Stanley, G. H. S. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.
(41) Fletcher, M. J. A Colorimetric Method for Estimating Serum Triglycerides. *Clin. Chim. Acta* **1968**, *22*, 393−397.
(42) Searcy, R. L.; Bergquist, L. M. A New Color Reaction for the Quantitation of Serum Cholesterol. *Clin. Chim. Acta* **1960**, *5*, 192−199.
(43) Prasad, P.; Savyasachi, S.; Reddy, L. P. A.; Sreedhar, R. V. Physico-Chemical Characterization, Profiling of Total Lipids and Triacylglycerol Molecular Species of Omega-3 Fatty Acid Rich B. Arvensis Seed Oil from India. *J. Oleo Sci.* **2019**, *68*, 209−223.
(44) Dalmia, A.; Tumaney, A. W. Optimisation and Evaluation of Cryopreservation Method for Aurantiochytrium Limacinum. *J. Appl. Phycol.* **2021**, *33*, 869−878.
(45) Marklund, S.; Marklund, G. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. *Eur. J. Biochem.* **1974**, *47*, 469−474.
(46) Aebi, H. Catalase in Vitro. *Methods Enzymol.* **1984**, *105*, 121−126.
(47) Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione S-Transferases. The First Enzymatic Step in Mercapturic Acid Formation. *J. Biol. Chem.* **1974**, *249*, 7130−7139.
(48) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(49) Socci, D. J.; Bjugstad, K. B.; Jones, H. C.; Pattisapu, J. V.; Arendash, G. W. Evidence That Oxidative Stress Is Associated with the Pathophysiology of Inherited Hydrocephalus in the H-Tx Rat Model. *Exp. Neurol.* **1999**, *155*, 109−117.
(50) Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. *Anal. Biochem.* **1979**, *95*, 351−358.
(51) Mesquita, C. S.; Oliveira, R.; Bento, F.; Geraldo, D.; Rodrigues, J. V.; Marcos, J. C. Simplified 2,4-Dinitrophenylhydrazine Spectrophotometric Assay for Quantification of Carbonyls in Oxidized Proteins. *Anal. Biochem.* **2014**, *458*, 69−71.
(52) Granger, D. L.; Taintor, R. R.; Boockvar, K. S.; Hibbs, J. B., Jr. Measurement of Nitrate and Nitrite in Biological Samples Using Nitrate Reductase and Griess Reaction. *Methods Enzymol.* **1996**, *268*, 142−151.

https://doi.org/10.1021/acsomega.2c00050
ACS Omega 2022, 7, 11914−11928