Characteristics of *Morus alba* L cultured by in-room hydroponics

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Abstract

Morus alba L, (cv Ichinose) was cultivated by an in-room hydroponics. The flavor and texture of leaves were markedly improved enough to be edible. When the contents of 1-deoxynojirimycin (DNJ) and polyphenoles were measured in the hydroponic cultivar, DNJ increased in the leaf compared to the field grown *M alba*. However, polyphenols, in contrast, decreased compared to the field cultivar. HPLC profiling revealed marked difference of leaf components between hydroponic and field cultivars indicating relative contents of lipophilic polyphenols were increased. The polyphenols contents, especially, lipophilic polyphenols in the root were remarkably high compared to So-Haku-Hi. The anti-obesity effect of the hydroponically grown Morus was further studied in rats by feeding high-fat, high-sucrose (HFHS) diet with and without supplementation of dried leaf and root powders for 15 weeks. As the results, both the leaf and root from the hydroponic cultivar showed potential anti-obesity and anti-hyperlipidemic functions through amelioration of insulin resistance. Differential effects of leaf and root powders indicated that besides DNJ, the lipophilic polyphenols may play a crucial roles in the anti-diabetic function of hydroponically grown *Morus alba L*. The hydroponics will provide an alternate way to cultivate a novel resource of Morus for developing functional foods and medicines.

Key words: Hydroponic culture, *Morus alba L*, DNJ, anti-diabetic function, functional manipulation

Introduction

The medicinal benefits of plant resources have been well recognized, and the functional application of traditional and new resources is attracting more attention in not only medicines but also in functional foods (**1, 2, 3**). One of the targets of herbal approach is the Type 2 diabetes, the complication characterized by decreased insulin sensitivity, that associates with hyperglycemia and hyperlipidemia, and is the major risk factor of many fatal diseases such as cardiovascular diseases, neural disorders, and cancer. Therefore, the prevention and treatment of type 2 diabetes are the worldwide issue (**4,**

5). There are several plant resources that have historically been used for treating diabetic conditions as discussed in several review articles (**2, 6, 7**). Among them, *Morus species (Mulberry)* is one of the most promised examples. The health beneficial functions of Morus are well known such that the dried root bark is a well-known oriental herbal medicine called So-Haku-Hi (Sang Bai Pi in Chinese), having anti-inflammation, diuresis and antitussive functions (**8**), and the leaf and twig have also been used in folk medicines such as in the forms of tea or decoction to treat diabetes-like symptoms (**9**). The anti-diabetic function of Morus was rationalized, since 1-deoxynojirimycin (DNJ), an inhibitor of β-glucosidases, was identified as the active principle (**10**). In addition to DNJ, many ingredients including characteristic polyphenols have been identified, and discussed their variety of functions besides anti-diabetic function (**11, 12, 13**). Therefore, health-beneficial importance of *Morus* in the functional foods and medicines are progressively recognized (**14, 15**).

However, *Morus* species are diverse and hardly defined genetically, although three types of Morus, *M. alba*, *M. rubla,* and *M. nigra*, are well known, those can be differentiated by the appearances of bark (**14**). Moreover, the contents and types of active ingredients are known to be variably changed among the species, and also depending on not only regional environmental factors such as climate, temperature, and sunlight, but also by the maturity and processing methods for the products (**16**, **17, 18**). Therefore, it is usually difficult to maintain the quality of *Morus* products prepared from the cultivar in the field.

The hydroponic culture, currently being used in the plant factory for cultivating vegetables and flowers (**19**), has an advantages to control the growing conditions such as temperature, lighting and nutritional conditions, and allows to harvest quality controlled products such as in terms of nutritional composition through the year. The advantages will be more apparent in the cultivation of herbal resources used in functional foods and medicines for manipulating active ingredients. However, challenges are few to grow herbal trees such as *Morus* (Mulberry) to date.

In the present study, we showed that *Morus* alba L having anti-metabolic syndromes function is able to be successfully grown by the hydroponic culture, and the cultivar had several advantages such as improved ingredient profile.

Materials and Methods

Hypotonic culture of *M. alba L*

Morus alba L (cv Ichinose) (MA) was grown in the hydroponic culture system developed by Morera Co. Ltd. (ex Stream Co Ltd.) in Nagano, Japan. The growing condition is briefly descried below. The seeds were germinated in a polyurethane foam for 30 days at room temperature 22^{o}C. The hatched MA (approximately, 1-2 cm high) was transplanted to the styrofoam board and set in the cultivating stand equipped with stainless steel pan in that water containing appropriate concentration of commercially available fluid fertilizer (Vegetable Life A, OAT Agrio Co.Ltd, Tokyo Japan) is continuously circulated, and then cultivated at 23 ^{o}C under illumination of 12hr light and dark cycle by white fluorescence lamps as shown in **Fig.1.**

Sample preparation

After 3 months cultivation of MA (HM) by the in-room hydroponics, each of leaf, twig and root were harvested, freeze-dried and powdered using ball-mil. The power was packed in vacuum aluminum bags and kept in a freezer at – 20 ^{o}C until use.

Practically, the leaves and twigs are harvested repeatedly from the tree maintained until 6 months, and the roots are harvested at 6 month, because the yield of fibrous root markedly reduced after 6 month. For the animal feeding experiments, air dried powders prepared from these samples were used.

Histological inspection of leaf tissue

The fresh leaves from HM and field grown MA (FM) were dissected into 5 mm in diameter pieces with a biopsy trepan (Kai corporation, Tokyo, Japan), fixed in the Farmer's fixative (ethanol:glacial acetic acid 3:1), dehydrated in a series of graded ethanol and embedded in paraffin after xylene substitution. The paraffin block is trimmed, sectioned by a microtome to obtain 10 μm thick sections to observe under microscopy after staining by 0.1% toluidine blue.

Quantification of 1-deoxynojilimycin (DNJ)

DNJ was quantified by LCMS as described elsewhere (**20**). Briefly, MA sample powders were extracted with water and the extract was separated on HPLC equipped with CAPCELL CORE PC-reverse phase column (Shiseido Co.,Ltd , Tokyo) using a gradient elution mode as follows: A 0.1% formic acid, B Acetonitrile. Gradient: B Conc. 80-40 % in 0-2 min, 40 % in 2-7 min, 80 % in 7-12 min, at flow rate of 0.2mL/min.The elution peak of DNJ was detected by MS using the condition given below: Ionization: ESI, Positive mode, Detection: MRM.

Total polyphenols and flavonoid determination

The MA powders were extracted with 70% aqueous MeOH and the contents of polyphenols and flavonoids were determined by Folin-Ciocalteu method (**21**) and Aluminum chloride method (**22**), respectively.

Briefly, to determine total polyphenols, 50 μL of MA sample extracts were diluted with 2.0 mL of distilled water and reacted with 500 μL of Folin-Ciocalteu's reagent (Merck KGaA, Germany) and then the absorbance was measured at 750 nm. Gallic acid was used to establish a standard curve and the results were expressed as gallic acid equivalent (mg/g dry sample).

To determine the total flavonoid content, 100 μL of MA sample extracts were reacted with 60 μL of 10% aluminum chloride solution for five minute, following addition of 1 mM sodium hydroxide and 70% ethanol, and then the absorbance was measured at 430 nm. Quercetin was used to establish a standard curve. The results were expressed as quercetin equivalent (mg/g dry sample). All the measurements ware triplicated.

HPLC profiling of leaf components

The MeOH extracts of HML and FML were separated on a reverse phase column

(phenomenex Gemini C18) equipped with Shimazu HPLC apparatus (LC-10ADvp) by the gradient elution mode with 0.1% formic acid/H2O and 0.1% formic acid/MeOH as elution solvents. The separated components were monitored by Shimazu SPD-M10A detector at 280 nm absorbance. Chrologenic acid (peak 2) was quantified using authentic sample as standard. Compound A eluted around at Rt=55 min (peak 12) was qualitatively compared as an example of lipophilic components from the peak hight ratio against Diosmetin used as the internal standard.

Long term feeding experiment in rats

Animals

Four-week-old male Wister/ST rats were purchase from SLC Japan (Shizuoka, Japan). Two rats were housed in one cage. All rats were kept under constant temperature at 22±1 ℃ and illumination with 12-h light/dark cycle, and had free access to water and experimental diet, during acclimation and long term feeding trial. After acclimation for one week with basal diet (Labo MR stock, Nihon. Nosan Kogyo, Shizuoka, Japan), rats were randomly grouped into four designed diet groups (n=8 in each group) described below.

Feeding groups and diets

Air dried powders of leaf containing stem and root of HM were used for feeding experiments. Four types of diets prepared for the feeding experiment are follows: Cont.; normal diet (AIN-93M), HFHS; high-fat high-sucrose diet, HML; HFHS diet supplemented with 5% (w/w) leaf powder (containing stem) of HM, and HMR; HFHS diet supplemented with 5% root powder (w/w) of HM. The nutritional composition of each diet was adjusted so as to match the total calorie with HFHS based on the nutritional component analysis data of leaf and root powders as the same way as reported elsewhere (**23**) (**Supplement, Table I and II**).

Casein, cornstarch, α-cornstarch, lard, beef tallow, cellulose powder, soybean oil, AIN-93M mineral mixture and AIN-93M vitamin mixture were obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan). L-Cystine and *t*-butyl-hydroquinone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan)

Experimental protocol

During the feeding trial for 15 weeks, body weight was recorded weekly, and the dietary intake volume was recorded every day. At the end of feeding trial, rats were starved overnight, anesthetized with pentobarbital, and the blood was corrected in a heparinized syringe from the subclavian veins for biochemical analysis. The blood plasma was separated by centrifugation at 3000 rpm and 4 ℃ for 5 min using a micro centrifuge apparatus (Sigma 1-14) and was stored in a freezer at -80 ℃ until use. At the same time, the tissues including mesenteric, epididymal, and perirenal fats, and liver were removed, rinsed with chilled saline, and weighed. A small pieces of liver tissue was sampled and fixed in 10% formalin neutral buffer solution for histological analyses, and the rest of the liver was stored at -80 ℃ until biochemical analysis.

The experimental protocol and animal treatment procedure were approved by the Niigata University of Pharmacy and applied life sciences and complied with the Guideline of Animal Care and Treatment (Approved No.2026-12).

Biochemical analysis

Plasma glucose, cholesterol and triglyceride were measured respectively using Glucose C II-test Wako, Cholesterol E-test Wako, and Triglyceride E-test Wako (Wako Pure Chemical Industries Ltd. (Osaka, Japan) according to the protocols attached, respectively. Plasma insulin was determined using LBIS Rat Insulin ELISA Kit (FJIFILM Wako Shibayagi Corporation, Gunma, Japan).

Liver lipids were extracted according to the method by Folch et al. (24),. Briefly, the liver was homogenized in AcOEt to extract lipids. After the solvent was evaporated off, the residual lipids were dissolved in 200 μL of 10% Triton-X 100/isopropanol, and measured triglycerides and total cholesterol as above.

Histological inspection of liver tissue

Fixed liver samples were dehydrated using an increased gradients of ethanol, embedded in paraffin, and then sectioned into 5 μm thick slices. The tissue slices were stained with Hematoxylin-eosin by standard procedure.

Statistic evaluation

All the data were expressed as mean ± SEM, and analyzed by Statcel 4 software (OMS Publishing Inc., Saitama, Japan). Statistical analysis was performed using the unpaired Student's t-test to mean of two groups, and one-way ANOVA followed by the Tukey-Kramer HSD test applied for comparisons between multiple experimental groups. Differences were considered statistically significant a p value < 0.05.

Results

Texture and histology of HM leaf

The hydroponic cultivation of *Morus alba L* (HM) apparently resulted in changing the **t**exture and taste of leaves such that the texture is enough soft and edible as a vegetable. Supporting this, the histological examination revealed the air boundary layer or cuticle of HM leaf looks thiner than FM. The spongy layer also is less stuffed compared to that of FM (**Fig. 2**).

Contents of 1-deoxynojilimycin and polyphenols in the leaf and root of HM

The contents of 1-deoxynojirimycin (1-DNJ), polyphenols were determined for the leaf (HML) and root (HMR) from *M. alba* cultured by the in-room hydroponics for 3 months and compared to those of field cultivars of MA. Since it is difficult to obtain the same fibrous root from FM, we could not obtain the data of 1-DNJ contents as well as polyphenols and flavonoids for FMR, and thus they were compared to So-Haku-Hi (mulberry root bark or Sang-Bai-Pi in Chinese) obtained from a Pharmacy store nearby, instead (**Fig 3**).

It was found that the 1-DNJ contents both in the leaf (HML) of hydroponic cultivar of *M. alba* and the leaf containing stem (HMLS) samples were apparently high compared to the leaves (FML) obtained from any of 3 different field cultivars examined. The contents in the root of HM is apparently higher than the leaf and also higher than So-Haku-Hi which level is almost same as HML.

On the other hand, the contents of total polyphenol and flavonoid (not shown) in HML and HMLS were almost half of the levels of FML found in the three different field cultivars of MA (**Fig. 4**). However, the polyphenols content of HMR was apparently high compared to any of FMLs and also So-Haku-Hi.

Although the data are not shown, there was a little difference in the polyphenols and flavonoids contents between HML and HMLS such that flavonoids content was higher in HMLS than HML.

HPLC profiling of Leaf components

HPLC profiling of components in 70% MeOH extracts of HML and FML showed marked difference (**Fig. 5**). The relative distribution of rather hydrophilic components was high in FLM but in contrast, lipophilic components were rich in HML. This is further exampled by comparing the relative amounts of chrologenic acid as typical hydrophilic polyphenols and compound A as an example of lipophilic polyphenols, respectively, in HML, HMLS and FML. Since the total contents of polyphenols are almost same, it is indicated the lipophilic polyphenols were relatively increased in HML.

Solvent extraction of polyphenols and DPPH radical scavenging activity

The polyphenols contents and DPPH radical scavenging activity was measured in the different solvent extracts of HMR (**Fig. 6**). Since many lipophilic polyphenols are identified as the characteristic ingredients of *Morus* (**12, 25**), the root powder was extracted with three different solvents, MeOH, AcOEt and H2O, and compared the polarity of existing polyphenols. The result showed that the highest distribution of polyphenols was found in the ButOH fraction followed by MetOH and H2O fractions. So-Haku-Hi also showed the same distribution profile but the content of lipophilic phenols was remarkably low compared to HM in all solvent fractions.

When DPPH radical scavenging activity was measured for each solvent extracted fraction, the highest activity was found in the ButOH extract both for HM and So-Haku-Hi. The DPPH scavenging activity was fairly correlated to the polyphenols content in these extracts from HMR, but the correlation was weak in the case of Ho-Haku-Hi, indicating the components related to the radical scavenging activity are different between HMR and So-Haku-Hi.

Our current studies on component analysis revealed that the relative contents of such polyphenols like oxyresveratrol, Morcin, and Kuwanons are markedly increased in the root as same as in the leaf shown in Fig. 4, compared to FM and So-Hakku-Hi (to be published elsewhere).

Anti-obesity function of HM

The body weight changes of rats were followed for 15 weeks by feeding the high fat high sucrose diet (HFHS) with and without supplementation of HML and HMR powders in **Fig. 7**. HFHS fed group showed greater body weight gain compared to the control group fed normal NAI diet during the feeding trial. However, the body weight gain was significantly suppressed by the supplementation of HMR powder to almost the same level as control group. The HML group also suppressed the body weight gain as well but the extent was small and not significant.

The weekly sum of diet intake during the feeding trial showed no differences between HML and HMR groups, although their levels were significantly low compared to control diet group (**data not shown)**. When the dietary intake volume was compared on the same total calorie base, no significant difference was observed among HFHS, HML and HMR groups, although they were higher than control diet group.

Effect of long term feeding of HM on Plasma glucose and insulin levels

Fasting blood sugar and insulin levels in the plasma after 15 weeks feeding trial were measured (**Fig. 8**). The blood sugar levels did not show significant differences among all test groups, but the insulin levels showed clear differences among them, such that the plasma insulin level was significantly high in HFHS group compared to normal diet control indicating the insulin resistance was acquired, but the insulin level was significantly decreased in both HML and HMR groups, especially in HMR group.

Effect of HM supplemented diet on visceral rat deposit

Marked differences in visceral fat volumes after 15 weeks feeding trial were observe among the different diet groups. (**Fig. 9**) The fat volumes of epididymal, perirenal and mesenteric adipose tissues were significantly increased in the rats fed HFHS but the increases were significantly inhibited in both HML and HMR groups. As same as the effect on the insulin resistance, HMR showed much stronger suppressive effects than HML on mesenteric ($P<0.01$), and epididymal ($P<0.01$) and perirenal ($P<0.05$) fat deposit.

Preventive effect of HM on fatty liver formation

In accordance with the increase of visceral fat volumes, histological observation indicated fatty liver pathogenesis in HFHS diet group after 15 weeks feeding trial. However, in HML and HMR supplemented diet groups, especially HMR group, the liver fat accumulation was apparently reduced and the parenchymal tissue images were significantly ameliorated (**Fig. 10)**.

Consistently, both Cholesterol and triglycerides content in the liver was markedly increased in HFHS group, but the increased accumulation of the neutral lipids was significantly decreased in HMR group. HML, however, did not give rise to significant effects on the liver fat accumulation (**Fig. 11**).

Discussion

Present studies revealed that the hydroponic culture technology can be adoptable to

Morus alba L (MA) that is well known medicinal tree having long history of using as folk medicine and traditional oriental medicines (**13, 14**).

Medicinal plant resources are attracting much attention as an alternative of western medicine, especially for treating complex diseases like diabetes and cancer (7, **25**). *Morus* species are the one having wide varieties of health beneficial functions usable in not only medicines but also functional foods or nutraceuticals (**26**). Although *Morus* leaf is rather accepted as the diet of silkworm (bombyx mori) than the food for human, the hallmarked benefit of this herbal tree is that all the parts including leaf, root, fruit and sting have medicinal importance. For example, dried root peel of MA named So-Haku-Hi (Sang-Bai-Pi) is not only an important component herb of several prescriptions in oriental medicine such as for treating airway diseases but also a major component of cosmetics having skin anti-aging and tyrosinase inhibiting activities (**8, 9, 26**).

The pharmacological and physiological functions of MA and related species have extensively discussed to date, those include antioxidant and anti-inflammatory functions, anti-obesity and anti-diabetes functions, anti-hyperlipidemia, anti-cancer, liver protective and neuroprotective functions, anti-viral function, anti-atheroscrerosis, hypoglycemic function, immune regulation, and tyrosinase inhibitory function and else (**27,28**), and thus *Morus* (Malberry) are implicated as the promised resource for functional foods and medicine.

However, the natural resources growing in the field have inherent disadvantages in terms of quality control, because the ingredients, thus the functional potential, are variable depending on the environmental factors such as soil, sunlight, and climate, and genetic hybridization (**16,17,18**). The field cultivation also has difficulty in avoiding contaminations by chemicals such as pesticides or soil originated toxic minerals. In contrast, in-room hydroponics has superiority to the field cultivation because safe and clean plant products can be harvested routinely throughout the year. It is not easy to compare the yield of leaf and twig of HM to the filed cultivation, but about 50 Kg of row leaf and twig mix per month can be harvested by the hydroponics in about 90 square meter space using the culture set up shown in Fig. 1. Another advantage is that the hydroponic culture allows to use the root as the simple functional resource for cosmetic and medicinal application, even for food. This is usually difficult for the root of *Morus* grown in the field.

Present study further indicated the health beneficial properties of MA can be manipulated by the hydroponic cultivation. For examples, the leaves acquired edible texture like vegetables for daily salads, as was supported by the histological examination. Moreover, it is noted that characteristic change of ingredient composition occurred in the HM, such that DNJ contents increased and the distribution profile of polyphenols changed both in leaf and root from FM. Especially in HMR, the polyphenol content was much higher than So-Haku-Hi, moreover, the composition of lipophilic polyphenols was remarkably increased compared to So-Haku-Hi. Moreover, the ingredients profiles of HM were not significantly changed during 3 to 6 month cultivation, that is, the lipophilic polyphenols were the major component.

These results indicates that the in–room hydroponic cultivation will provide a new strategy to fortify the active ingredient composition of MA.

Anti-diabetic effect is most extensively discussed in MA because the hypoglycemic effect has been implicated as the typical function of *Morus* species from ancient period (**29**), and the increase of obesity related type 2 diabetes became social issue worldwide especially in developed countries (**30**). Indeed, 1-deoxynojirimycin (DNJ) as glucosidase inhibitor was reported as the active principles for the anti-obesity effect of *Morus* species (**10**). Now, the hypoglycemic effect of MA has become obvious from many studies including *in vitro*, animal systems, and even clinical human trials (**29, 31**). Besides DNJ, several lipophilic polyphenols also have potential to inhibit glucosidase activity (**32**), suggesting that the anti-obesity or anti-diabetic function of *Morus* is an integrated functions of DNJ and other active components.

The present study also suggested beneficial use of HM, especially the root, against diabetic symptoms. As was expected by the presence of 1-DNJ, both HML and HMR prevented HFHS diet induced obesity. The marked anti-obesity function of HMR suggested the synergy effects of DNJ and other ingredients, probably lipophilic polyphenols. Consistently, both HML and HMR inhibited visceral and liver fat accumulation, but their effects were markedly differed. Visceral fat accumulation in all three tissues was more effectively inhibited by HMR than HML. It is interesting to note, in liver, HMR apparently reduced fat deposit but HML's effect was almost none, indicating the mechanism, thus the involved components in manipulation of lipid metabolism, is different between HMR and HML. It was also noted that although the anti-obesity potentials differed, both HMR and HML supplementation ameliorated the insulin resistance acquired by HFHS diet, suggesting the lipophilic polyphenols as antioxidant may play crucial roles in the anti-diabetic functions of MA, especially of HM, since the relative contents of lipophilic polyphenols was also high in HML as shown in Fig. 5. These data strongly suggested potential roles of lipophilic polyphenols in the health beneficial functions of *Morus*, and the hydroponic culture will possibly fortify theses functional ingredients.

Although the data are not shown, the lipid excretion in the feces, both cholesterol and triglycerides, was accelerated by the leaf better than the root. This will suggest that accelerated excretion of lipids, especially triglyceride contributed to the anti-obesity function of HM and the leaf contains some other component having such as lipase inhibiting or intestinal microbial flora modulating activity, other than lipophilic polyphenols rich in the root. Taken all together, the mechanism or the component involved in the anti-obesity effect of HM is probably different between the root and leaf. Further studies are necessary to identify the component involved.

In conclusion, the in-room hydroponic culture of MA reported here suggested an alternate way to provide high quality resource of MA that is usable not only as vegetable but also for developing functional foods and medicines.

Conflict of Interest

Stream Co.Ltd., collaborated to develop the hydroponic culture system of *Morus alba L,* and provided experimental samples for the present studies.

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Table and Figure legends

Tabe I: Nutritional Composition of HMA and Supplemented diets for animal experiment

Fig 1: In-room hydroponic culture of *Morus alba L* (Ichinose)
MA was grown by in-room hydroponics for 3months after seeding.

Fig 2: Differences of leaf architecture of in-room hydroponic culture and field grown *M. Alba.*
The cross sections of fully matured leaves harvested from HM and FM were investigated under a microscopy.

Fig 3: 1-DNJ contents in of *M.alba* cultivated by in-room hydroponics and in the field.
Water extract of HML powder was analyzed by LCMS to quantitate 1-DNJ as described in Method section, and the value was compared to those of three different FMLs, So-Haku-Hi and HMR. Three different FML1-3 are from field grown M. Alba L (Vr Ichinose, Vr Nakajima and name unknown, respectively). SHH is So-Haku-Hi. Data are presented as Mean +/- SD (n=3).

Fig 4: Polypenols contents of *M. Alba* cultivated by in-room hydroponics and in the field.
Total polyphenols was determined for MeOH extracts as described in method section. Data are presented as Mean +/- SD (n=3)

Fig.5: HPLC profiles of leaf components of hydroponic culture and field grown *M. Alba*.
The 70% MeOH extract of leaves are separated by a reverse-phase HPLC using gradient of formic acid /formic acid-MeOH as elution solvent. A: Field cultivar, B: hydroponic cultivar. Chrologenic acid: Peak 2, Compound A: peak 12. Data are given as Mean +/- SD (n=3)

Fig 6: Distribution of polyphenols and antioxidant activity in different solvent extracts of in-room hydroponic culture of *M. Alba*.
HMR and So-Haku-Hi were extracted with MeOH, AcOH and H2O, respectively and the contents of total polyphenols and DPPH radical scavenging activity were determined as described in method section.Data are presented as Mean +/- SD (n=3)

Fig 7: Body weight change during HFHS diet feeding trial with and without HM supplementation
Rats were fed for 15 weeks with HFHS diet with and without supplementation of HMA leaf or root powders.

Fig 8: Fasting plasma glucose and insulin levels after 15 weeks feeding trial
After 15 weeks feeding trial of HFHS diet and the HMA supplemented diets, fasting

glucose and insulin levels were determined.

Fig9: Effect of HM supplement on visceral fat accumulation.

After 15 weeks feeding trial, visceral fat tissues were removed and wighted their volume.

Fig.10: Preventive effect of HM on fatty liver formation

After the long term feeding trial, the liver tissue was removed, washed in the chilled saline, and photographed. A piece of the liver tissue was fixed, with formaldehyde, sliced and histologically observed after fixation with Hematoxylin-Eosin.

Fig.11: Effect of HM supplementation on neutral lipid accumulation in liver .

After 15 weeks feeding trial, liver was removed and the lipids were determined as described in method section.

Table and Figures attached.

Supplement tables

Table I: Nutritional composition of HM leaf and stem

/100g	Leaf	Root
Energy	251	249
Moisture (%)	6.2	5.1
Protein (g)	22.6	18.1
Lipids (g)	4.0	3.5
Carbohydrates (g)	53.3	64.8
Sugar (g)	9.0	7.6
Fibers (g)	44.3	57.2
Ash (g)	13.9	8.5
Na (mg)	2.0	22.0

Table II: Diet formula for long feeding experiment

Composition	Ingredients	Cont (AIN-93M)	HFHS	HML	HMR
Priotein	Casein	14.0	25.0	23.9	24.1
Carbohydrate	Corn starch	46.6	0.0	0.0	0.0
	α-Corn starch	15.5	14.9	14.4	14.5
	Sucrose	10.0	20.0	20.0	20.0
Fiber	Cellulose	5.0	5.0	2.8	2.1
Lipids	Soy lipids	4.0	2.0	1.8	1.8
	Lard	0.0	14.0	14.0	14.0
	Beef tallow	0.0	14.0	14.0	14.0
Vitamin mix		1.0	1.25	1.25	1.25
Mineral mix		3.5	3.5	3.5	3.5
L-Cystine		0.18	0.375	0.375	0.375
t-Butyl hydroquinone		0.0008	0.006	0.006	0.006
MA sample	Leaf or Root	0.0	0.0	5.0	5.0
					g/100g

Fig 1: In-room hydroponic culture of *Morus alba L* (Ichinose)

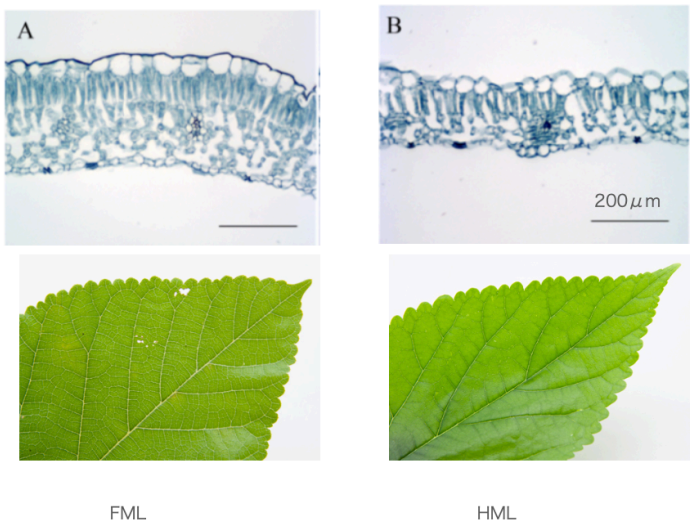

Fig 2: Differences of leaf architecture of in-room hydroponic culture and field grown *M. Alba.*

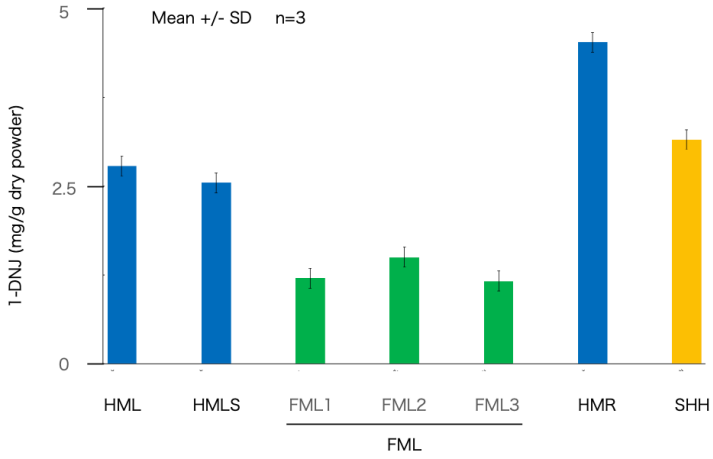

Fig 3: 1-DNJ contents in of *M.alba* cultivated by in-room hydroponics and in the field.

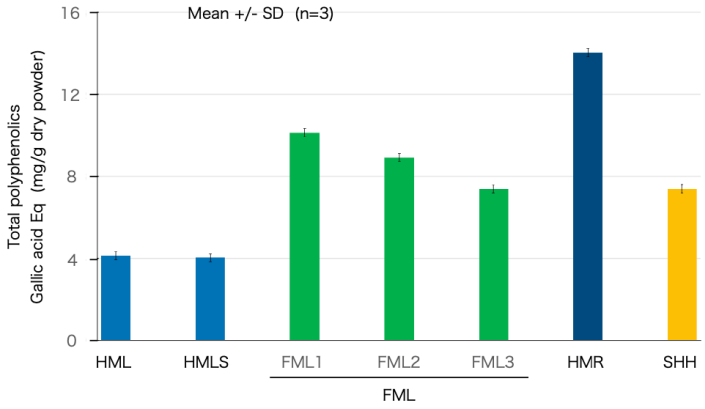

Fig 4: Polypenols contents of *M. Alba* cultivated by in-room hydroponics and in the field.

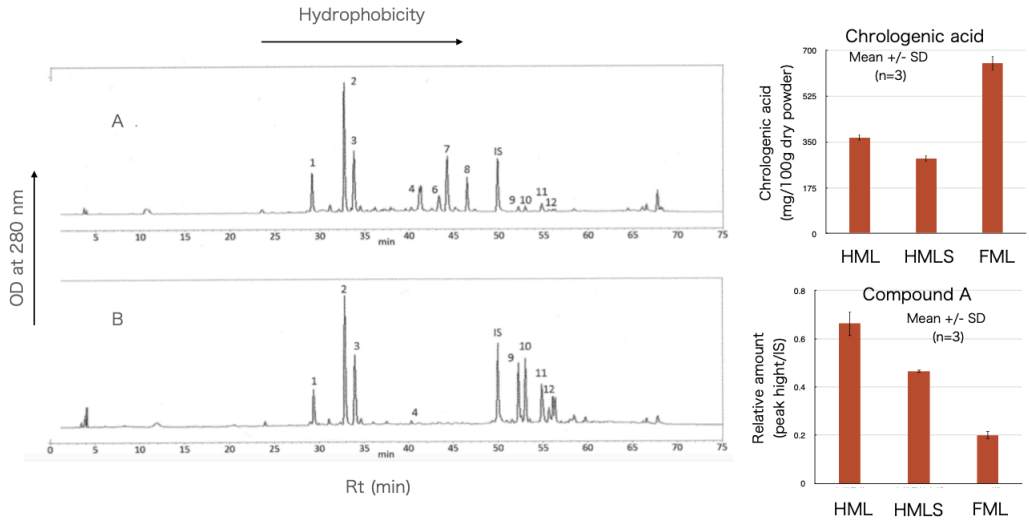

Fig.5: HPLC profiles of leaf components of hydroponic culture and field grown *M. Alba*.

Chrologenic acid: peak 2, Compound A: peak 12

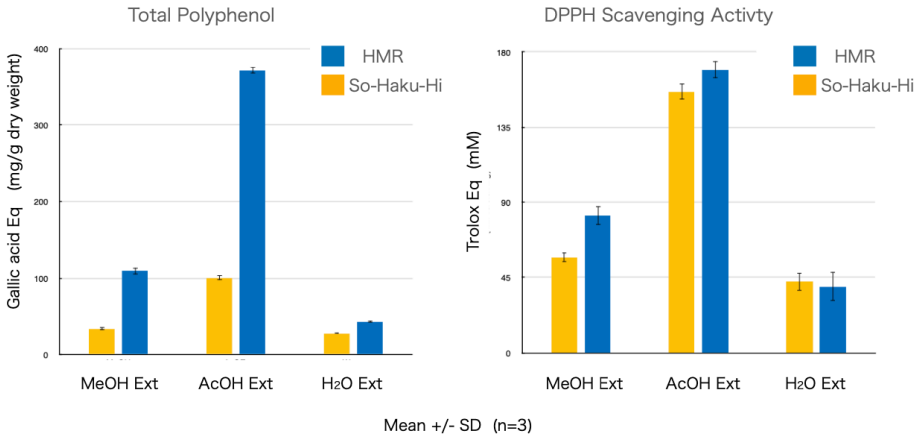

Fig 6: Distribution of polyphenols and antioxidant activity in different solvent extracts of in-room hydroponic culture of *M. Alba*.

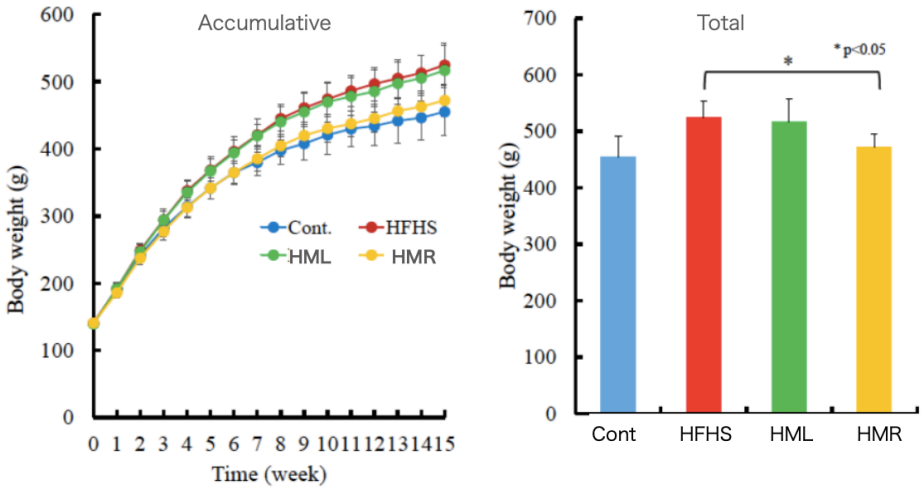

Fig 7: Body weight change during HFHS diet feeding trial with and without HM supplementation

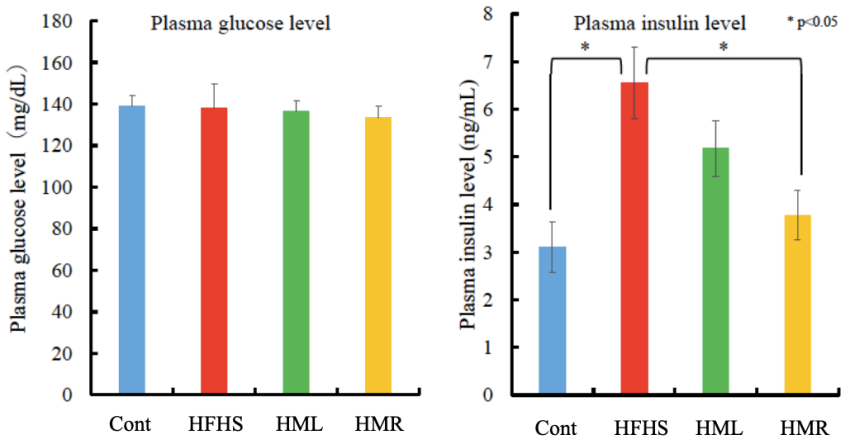

Fig 8: Fasting plasma glucose and insulin levels after 15 weeks feeding trial

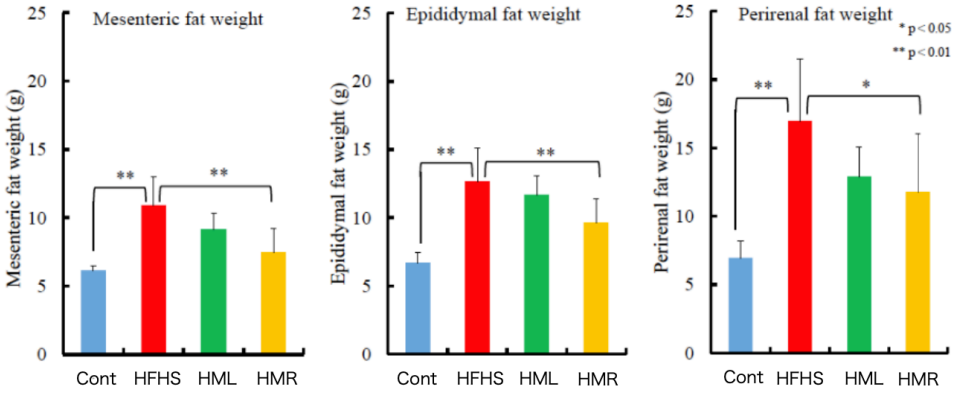

Fig9: Effect of HM supplement on visceral fat accumulation.

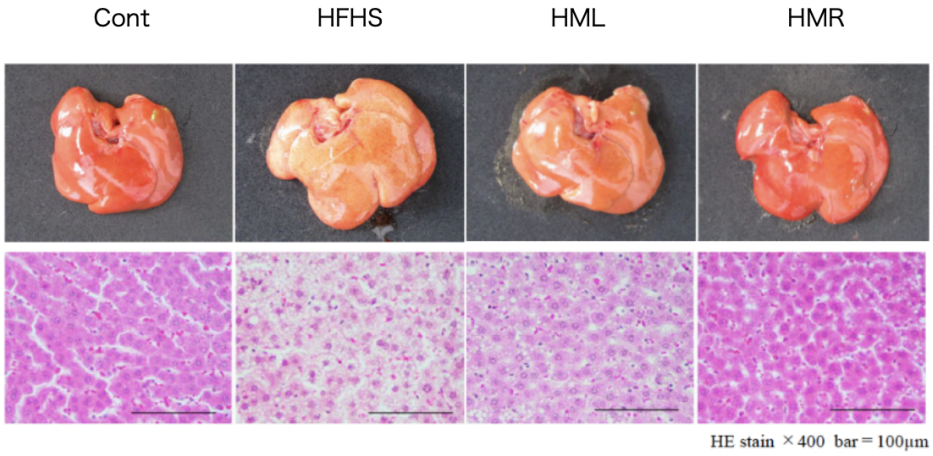

Fig.10: Preventive effect of HM on fatty liver formation

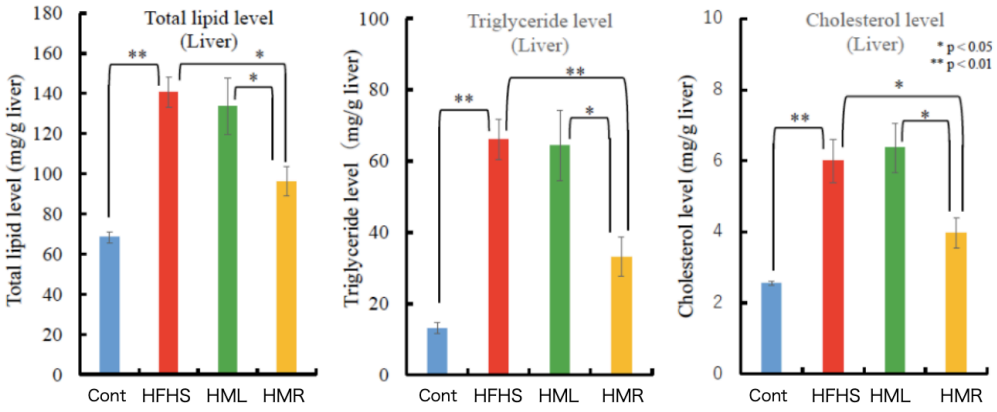

Fig.11: Effect of HM supplementation on neutral lipid accumulation in liver