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Study on Preparation and Bioactivity of Peony Wood Vinegar

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Abstract

Wood vinegars without secondary pollutants are extracted from plant material . Recently, research on wood vinegars has received increasing attention because of the rising awareness of environmental protection. Herein, we first exploited the wood vinegar obtained from peony which has never been discovered. This wood vinegar liquor was extracted by dry distillation and collected with four different methods which included different temperature sections of 0 ~ 110 °C, 110 ~ 150 °C and different attached containers of condenser pipe, flask (M_1, M_2, M_3 and M_4 respectively). The chemical compositions of every segment were analyzed by gas chromatography-mass spectrometry (GC-MS). In addition, the antioxidant activities of DPPH

free radical and superoxide anion detection were investigated. We also developed the anti-inflammatory activity of every dry distillation with RAW 264.7 cell line. The result showed that dry distillations collected at two different temperature have a great antioxidant activity compared with vitamin C. Meanwhile, it revealed a powerful anti-inflammatory on these two dry distillations. This research provided a novel research direction for high-value utilization of waste plants on peony, which can be used as food additives or drugs in the future.

Keywords: *Peony; Wood vinegar; Dry distillation; Antioxidant activity; Anti-inflammatory activity*

1. Introduction

Wood vinegar is an organic liquid obtained through condensation recovery and separation of the gas produced by biomass during its pyrolysis carbonization process[1].The source distributions of wood vinegar were widespread, and the wood vinegar is easy to be obtained the branches, trunk, nuts, kernel, sawdust, corn cob and other agriculture and forestry waste with low cost [2, 3]. Nowadays researchers are paying more and more attention to dealing with the environmental crisis, so there is an increasing interest in finding green methods which can naturally produce substances such as wood vinegar, wood hydrolysates without environmental pollution. Wood vinegar is also called liquid smoke or pyroligneous acid, which processed by high-temperature carbonization of wood with oxygen absence[4]. It is obtained from different materials which were primarily composed of acids, ketones and phenolic compounds. Some studies also discovered that wood vinegar had several positive effects on soil sterilization and disinfection, promoting the growth of plants, etc.[5-8]. Thus, wood vinegar can not only promote the economic vitality, but also protect the environment.

In the perspective of development and utilization of plant resources, the retorting (isolating the air and strengthening the heat) method is an effective way to acquire plant products (carbon, plant vinegar, etc.) [4, 9]. It varies greatly depending on the materials, methods, temperature and time of collecting distillate[10]. The main preparation method to obtain the crude wood vinegar in the laboratory is by retorting. Wood vinegar is a kind of auburn liquid with smell after condensing and separating the vapor gas mixture from the wood distillation equipment, which is a mixture of alcohol, phenol, ketone, aldehyde and its derivatives, mainly acetic acid. The components contained in the wood vinegar exhibit high antimicrobial activity

and powerful antioxidant activity[11-13]. Furthermore, many phenols from wood vinegar can be used as intermediates in the synthesis of pharmaceutical products, as well as food additives because of their germicidal activity and anti-diarrheal proprieties[14-18].

Wood vinegar reportedly improved soil quality, eliminates pests, accelerates plant growth as a plant growth regulator or growth inhibitor. So, it is significant to explore other functions or new effective wood vinegars. In this paper, we first investigated the wood vinega obtained from peony which never been researched. The wood vinegar from peony was extracted by dry distillation and collected with different methods whose chemical compositions of every segment were analyzed by GC-MS. Meanwhile, we investigated the antioxidant activities of DPPH free radical and superoxide anion detection. To study the biological application of peony wood vinegar, we also tested the cytotoxicity and the anti-inflammatory activity of every dry distillation with RAW 264.7 cell line. This study indicated the peony wood vinegar dry distillation under different temperature ranges of 0 ~ 110 °C, 110 ~ 150 °C have great antioxidant and anti-inflammatory effect, and no toxicity in a range of concentrations. These findings offered new perspectives for future value investigation on peony waste

2. Materials and Methods

2.1. Materials

All chemicals and solvents were used as received unless otherwise stated. Ultrapure water was used with a Millipore water ultrapurifi-cation system (Millipore, Bedford, MA, USA). HPLC-grade methanol and acetonitrile were purchased from the Fisher Company (Fairlawn, NJ, USA). The pH values were measured using a pH-3 digital pH meter. Ethanol, tris, pyrogallol, and HCl were purchased by China National Pharmaceutical Chemical Reagent Co. Ltd. DMSO, MTT, vitamin C and DPPH were bought from Sigma. Peony products were obtained from Heze Yao and Shun Penoy Compamy.S0021 NO detection kits were bought from Beyotime Biotechnology Company. RAW 264.7 cells were purchased from the Committee on Type Culture Collection of the Chinese Academy of Sciences.

2.2. Preparation of Peony Wood Vinegar

The wood vinegar liquor of peony was extracted by dry distillation and collected with four different methods which included different temperature sections of 0 ~ 110 °C, 110 ~ 150 °C and different attached containers of condenser pipe, flask (M_1, M_2, M_3 and M_4 respectively).

Then, these four kinds of products were settled for 3 months, and acetic acid evaporated in this process. The intermediate layer clarification solution was siphonized, then 5% activated carbon was added and ultrasonic oscillation was performed for 10 min, last step was to set the solution for 36 h.

2.3. Chemical Composition Analyzed by GC-MS

An appropriate amount of refined peony wood vinegar was dissolved in methanol, and the chemical components of the above M_1, M_2, M_3 and M_4 were analyzed by GC-MS [19-21]. According to MS (mass spectra) standard by NIST library (Muriel 1994). The method of determination was as follows, gas chromatographic conditions: the capillary column (30m×0.25mm×0.25 m), the inlet temperature was 220 °C, the column temperature was 60 °C, and the temperature was set at 6.0 °C/min, rising to 240 °C, and the temperature was kept at 8min.Shunt injection was 80:1, the carrier gas flow rate was 110 mL/min; MS conditions: EI source, the MS was carried out in the electron impact mode (EI) at 70 eV, ion source temperature was set at 250 °C, and the mass scanning range was from 35 ~ 400. With the assistance of database information and the data store software XCALIBUR, the name of the compounds of the experimental materials, molecular weights and structures were determined.

2.4. Evaluation of Antioxidant Activities

DPPH• (2, 2-diphenyl-1-picrylhydrazyl) is a stable and color-free radical which is well known as a good method to determine antioxidant capacity [22-24]. DPPH• is soluble in organic solvents and commercially available with a typical absorption band at 517 nm. For the DPPH• assay, we dissolved 12.5 mg of DPPH• in a solution of ethanol (250 ml) to prepare for serial dilution. 0.5 ml samples with varying concentration (0, 0.02, 0.04, 0.06, 0.08, 0.1mg/ml and 0.1, 0.2, 0.4, 0.6, 0.8 1.0 mg/ml) were added to the same 2.5 ml of DPPH• ethanol solution (100 μM). Mixtures were shaken vigorously and remained to incubate for 30 min in the dark at room temperature. A decrease in absorbance was measured at 517 nm against a blank of ethanol without DPPH• by using a microplate reader. The concentrations were measured for the vitamin C (control solution) range at 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml. A0 was the absorbance measured with the DPPH, A1 for control solution and A2 for samples. Clearance calculation formula:

$$SR\% = \{1 - [A_0 - (A_2 - A_1)] / A_0\} * 100\% \quad (1)$$

Superoxide anion (O2•−) is another text index to evaluate the antioxidant capacity by using the pyrogallol autoxidation method. In this part, 1.5 ml Tris-HCl buffer solution with pH 8.2

was prepared to add with the samples, the concentration ranges were same as DPPH• detection above, then we mixed 0.5 ml 30 mmol/L of catechol solution to react for 20 minutes compared with adding water. A decrease in absorbance was measured at 320 nm against a blank of water (A0). A1 was identified for without pyrogallol and A2 for adding pyrogallol. Clearance was calculated according to the following formula:

$$SR\% = (A_2 - A_1) / (A_1 - A_0) * 100\% \quad (2)$$

2.5. Cell culture

RAW 264.7 cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 100 U/mL of 1 % antibiotics penicillin / streptomycin and 10 % fetal bovine serum that maintained at 37 °C in a 100 % humidified atmosphere containing 5 % CO2 / 95 % air (20 % O2) for normoxic in incubator MCO-15AC (Sanyo, Tokyo, Japan).

2.6. Cytotoxicity assay

The cytotoxicity of peony wood vinegar with different concentrations (0, 25, 50, 100 μg/mL) was evaluated by the standard MTT assay [25]. All the samples were dissolved with DMSO. RAW 264.7 cells were cultured in 96-well microtiter plates and incubated in 5 % CO2 at 37 C for 24 h. After the original medium was removed, the cells were incubated with the samples (0, 25, 50 and 100 μg/mL) for 12, 24 and 48 h respectively. Next, 150 μL of MTT solution (0.5 mg/mL) was added to each well. The remained MTT solution was removed after 4 h, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. We used the microplate reader to measure the absorbance at 490 nm. The experiment was repeated three times. Viability was calculated based on the absorbance and the data were shown as the mean ± SD.

2.7. Evaluation of Anti-inflammatory capation

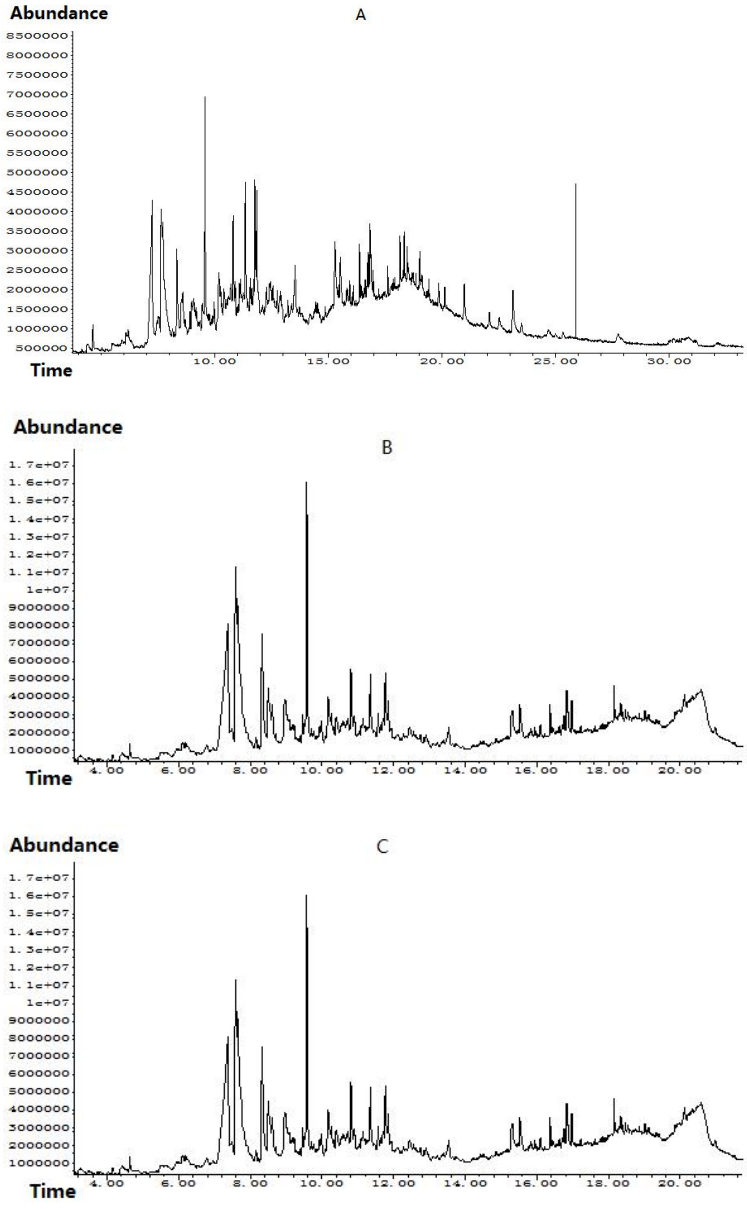
The mouse macrophage RAW264.7 cells were induced by lipopolysaccharide to establish an inflammatory model [26]. The effect of different treatment conditions on NO production was investigated with Griess method, then the anti-inflammatory activity of peony wood vinegar was evaluated. The cells in 96-well that have a good growth states were randomly divided into the control group (LPS, 1 mg· L^{-1}) and the experimental group (concentration gradient of wood vinegar solution was 0, 12.5, 25, 50 μg/L). We added 150 μL medium to incubate for 24 h, then removed the medium and added 100 μL of the medium with samples to culture 24 h for reaction. Next, 50 μL of Griess Reagent I and Griess Reagent II was added, and the absorption at 540 nm was measured with the microplate reader. The concentration of NO was

calculated according to the standard curve method and the indication on the NO assay kit. The experiment was repeated three times. After comparing to the solvent, we calculated the inhibition ratio and the data were shown as the mean ± SD.

3. Results and Discussion

3.1. Chemical Composition Analyzed by GC-MS

Here, we extracted the wood vinegar from peony by dry distillation and collected with different temperature ranges at 0 ~ 110 °C (M_1), 110 ~ 150 °C (M_2). Different attached containers of condenser pipe (M_3), flask (M_4), the chemical compositions were fully characterized by GC-MS which were shown in Figure 1.



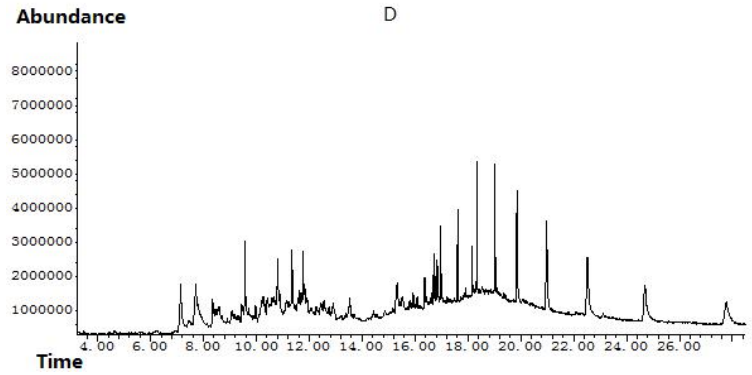


Figure 1 The chemical composition characterized by GCMS: (A) M_1, (B) M_2, (C) M_3, (D) M_4.

The absence of acetic acid in the composition was due to the deposition of the sample for three months. The contents of the organic fraction of peony wood vinegar which included different temperature sections of 0 ~ 110 °C, 110 ~ 150 °C and different attached containers of condenser pipe, flask (M_1, M_2, M_3 and M_4 respectively) were markedly different. As shown in Table 1, the separation of product M_1 and M_2 in peony wood vinegar has similar contents. The acids and polyphenol were the main contents in M_1 and M_2 while they were much less in M_3 and M_4, which showed that there were 18.453% acids in M_1, 17.716% acids in M_2, 14.400% in M_3 and 5.543% in M_4. Moreover, M_2 contained about 46.035% polyphenol whereas M_1 had about 32.416%, M_3 contained about 18.294% and 21.637% polyphenol in M_4, which indicated that M_2 had the strongest antioxidant activity. As we can see in Table 2, the alkanes (52.698%) rate was highest in M_4 while other three compounds (22.428% in M_3, 0% in M_2 and 2.285% in M_1) have much less alkanes, which indicated that M_4 had the lowest activity.

Table 1. The organic compounds of peony wood vinegar: (A) M_1, (B) M_2, (C) M_3, (D) M_4.

Type of Compounds	Substance	Concentration (%)			
		A	B	C	D
Acids		18.453	17.716	14.400	5.543
	Benzoic acid	14.932	17.716	8.406	5.543
	Homovanillic acid	3.521	--	--	--
	Palmitic acid	--	--	2.485	--
	Stearic acid	--	--	3.509	--
Polyphenol		32.416	46.035	18.294	21.637
	Catechol	26.439	36.052	14.569	9.736

	Phenols	5.987	9.983	3.725	10.418
	Maltol	--	--	--	1.483
Esters		12.281	-	4.785	--
	Methyl formate	6.309	--	--	--
	Phenyl ester	4.786	--	--	--
	Lactone	---	--	4.785	--
	Ethyl propionate	1.186	--	--	--
Alkane		2.285	--	22.428	52.698

3.2. Evaluation of Antioxidant Activities

The absorbance values of the four sample solutions and a control solution were measured by ultraviolet spectrophotometry at 517 nm. The inhibition percentage of DPPH• discoloration was calculated with formula (1). The inhibition percentages were shown in Figure. 2. We can see that the antioxidant capacity of M_1 and M_2 was strong, while the other two were not good. IC_{50} value is a significant standard for antioxidant activity. The smaller the IC_{50} value means the better the antioxidant activity. We also calculated the IC_{50} of each sample and control solution. As shown in Table 2, the antioxidant capacity ranked as follows: vitamin C, M_1, M_2, M_3 and M_4.

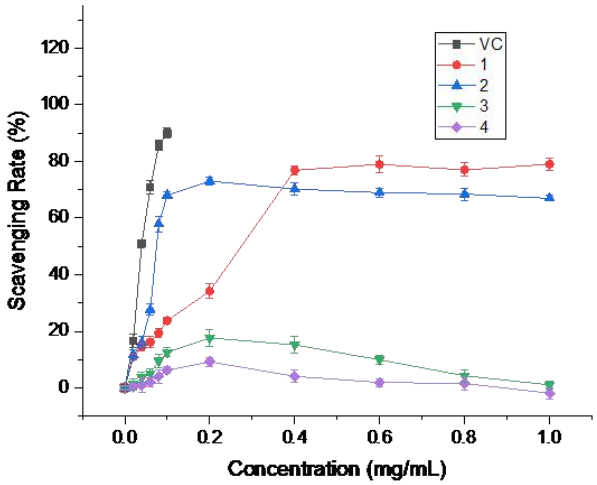


Figure 2. The DPPH• scavenging rate with M_1, M_2, M_3, M_4 and vitamin C.

Table 2. DPPH and superoxide anion ($O_2^{\bullet-}$) antioxidant activities of M_1, M_2, M_3, M_4 and vitamin C.

Samples	DPPH (IC_{50}, mg/mL)	$O_2^{\bullet-}$ (IC_{50}, mg/mL)
1	0.09	0.4
2	0.2	0.3
3	∞	∞
4	∞	∞
Vitamin C	0.06	0.06

Next, the superoxide anion ($O_2^{\bullet-}$) detection part was measured at 320 nm against a blank of water (A_0). The scavenging rate was calculated according to the equations (2). The data was exhibited in Figure 3. We can know that the scavenging rate increased fast of M_1 and M_2 at the concentration ranging from 0 to 0.4 mg/ml, then the rates tended to stay at the same level. It has a same trend that the antioxidant abilities of M_1 and M_2 was stronger than M_3 and M_4. IC_{50} values of superoxide anion were calculated to show the antioxidant activity. As shown in Table 2, the antioxidant capacity followed the order vitamin C>M_2>M_1>M_3>M_4.

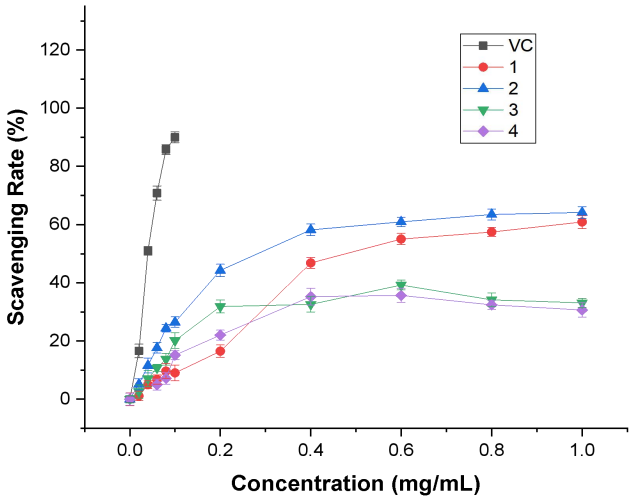


Fig. 3. The superoxide anion scavenging rates with M_1, M_2, M_3, M_4 and vitamin C.

3.3. Cytotoxicity Experiment

To evaluate the cytotoxicity of the peony wood vinegar, we performed an MTT assay in cell line RAW 264.7. The absorbance of MTT at 490 nm depended upon the degree of activation

of the cells. As shown in Figure 4, the peony wood vinegar had no obvious effect on RAW 264.7 cell viability for up to 48 h. These results indicated that the peony wood vinegar exhibited low bio-toxicity or side-effects on living cells.

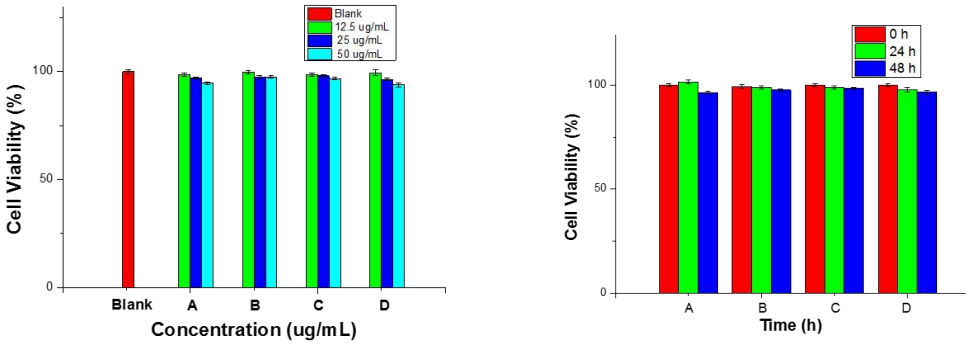


Figure 4. The viability of M_1 (A), M_2 (B), M_3 (C) and M_4 (D).

3.4. Anti-inflammatory Activity

The effect of peony wood vinegar on NO production in LPS-induced RAW 264.7 cells was tested to investigate the anti-inflammatory effects. The amount of nitrite accumulated in the culture medium was estimated by Griess reagent as index for NO. After being treated with LPS, the nitrite concentration in the medium increased much higher compared with the control group. As shown in Figure 5, with LPS simulation with peony wood vinegar intervention, the concentration of NO decreased more obviously than that with LPS only. It indicated that M_1, M_2, M_3 and M_4 all had a sound anti-inflammatory activity.

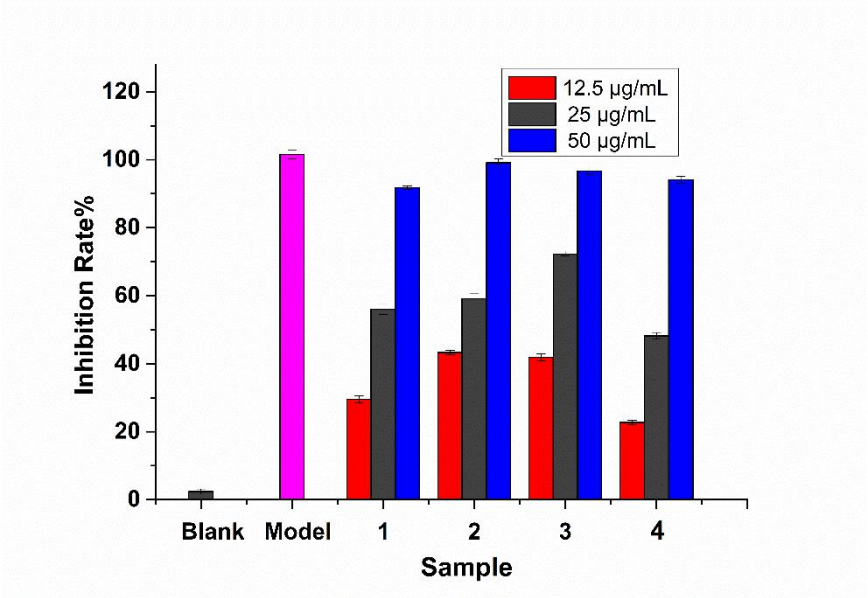


Figure 5. The anti-inflammatory activity of M_1, M_2, M_3 and M_4.

4. Conclusions

In summary, we developed a new wood vinegar extracted from peony. This peony wood vinegar was extracted by dry distillation and collected with four different methods which included different temperature sections of 0 ~ 110 °C, 110 ~ 150 °C and different attached containers of condenser pipe, flask. These compounds extracted with different temperature sections have a generally better viability, antioxidant and anti-inflammatory than those with attached containers of condenser pipe or flask. The results indicated that peony wood vinegar had a great research value to avoid environmental pollution. This will provide a potential environmentally friendly technologies from plant resources.

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