Original Article

Xanthine Oxidase and Lipid Peroxidation Inhibition of Taiwan Folkloric Medicine Factors Affecting *Rhus semialata* var. *Roxburghiana* Activities against Xanthine Oxidase and Ferrous Iron-induced Lipid Peroxidation on Mice Liver Mitochondria

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ABSTRACT

Background: Gout, a disease characterized by recurrent inflammatory for urate deposit from overactive xanthine oxidase, is common among Taiwanese. *Rhus semialata* var. *roxburghiana* (RSR) grows wildly in Central Mountain of Taiwan and used by aboriginal Taiwanese as traditional medicine for gout and hepatitis. **Methods:** In this study, the factors such as variations in RSR extracts in terms of geographical sources, aerial parts and extraction solvents were investigated for antioxidant capabilities, phytochemical analysis and inhibitory effects against xanthine oxidase and lipid peroxidation using ferrous ion as ROS inducer on mice liver mitochondria. **Results:** The results showed that ML-*n*-hexane and NS-acetone gave the maximum XO inhibitions ($IC_{50} = 16.74 \pm 0.74$ μg/mL) and LPO inhibitions ($IC_{50} = 8.40 \pm 0.35$ μg/mL) in the liver mitochondria of mice, respectively. And ML-*n*-hexane and NS-acetone contained phenolic and flavonoids compounds for its potential target components against XO and LPO in this study. **Conclusion:** Overall, RSR could lead to a medicinal potential as a suitable candidate for the development of a natural anti-gout and liver damage protective agent that have yet to be conducted.

Key words: *Rhus semialata* var. *Roxburghiana* extracts, Geographical source, Xanthine oxidase inhibitor, Free radical scavenging, Liver mitochondria LPO inhibitor.

INTRODUCTION

Gout is a disease characterized by recurrent inflammatory due to uric acid deposit in joint or tissue.[1] An important enzyme of Xanthine oxidase (XO) in the metabolism of hypoxanthine to xanthine and then to uric acid and produces reactive oxygen species (ROS), such as superoxide and hydrogen peroxide in biological systems[2,3] that normally forms chain reactions via lipid peroxidation, which have been proved to be the main cause for the oxidative damage and aging of the cells. Overactive XO leads to deposition of urate monohydrate in joints and tissues causing gout[4,5] and some pathogen etic condition, such as vascular injury, inflammatory disease,

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chronic heart failure and ischemic injury related to ROS damage.[6] Commercially available medicines for gout such as allopurinol-a well-known XO inhibitor, benzbromarone-a uricosuric drug, nonsteroidal anti-inflammatory drugs (NSAIDs) and colchicine, have adverse side effects including liver necrosis due to allopurinol,[7] and hepatotoxicity of benzbromarone,[8] NSAIDs and colchicine.[9,10] In this regard, continuous screening of potential natural source with antioxidant ability that balanced ROS to lessen lipid peroxidation of polyunsaturated fatty acids forming carcinogenic malondialdehyde (MDA) leading to cell injury or several pathological conditions.[11,12]

Taiwan is an island home to many kinds of native ethnobotany for folkloric medicine. Aboriginal Taiwanese such as Bunun tribe and Atayal tribe practice traditional medicine as treatment to some diseases using natural sources grown in their area. With the relatively high population of these aboriginal Taiwanese (11.5%), many of their men suffers severe (15.6%) hyperuricemia (serum level > 7 mg/100 mL) making Taiwan one of the countries with high gout population in the world (prevalence: 0.16 – 0.67%).[13]

Rhus semialata var. *roxburghiana* (RSR) (Anacardiaceae) is wildly grown in Taiwan Central Mountain from thickets and secondary forests at low altitudes in middle and north of Taiwan.[14] This plant has been reported to remedy diseases such as gout and hepatitis with high potential antioxidant and polyphenol[15] as well as pharmacological activities including anti-diarrheal activity,[16] antithrombin capability,[17] pBR322 DNA plasmid breaking ability and anti-tobacco mosiac virus.[18,19] On the other hand, The secondary metabolites or crude extract have been found from RSR such as phenolics, flavonoids and hydrolysable tannins etc. These compounds or crude extracts also have inhibited antioxidant, antimicrobial and antiviral activities etc. Overall, we summarize in Table 1 that the Biological and pharmacological properties of compounds or crude extracts from RSR.[20]

RSR has been used for a long time as folkloric medicine, albeit scientific studies proving its efficacy to some diseases are limited. This study aimed to investigate the factors such as variations in geographical source (Middle and Northern Taiwan), aerial parts (stem, branch and leaf) and extraction solvents (*n*-hexane, ethyl acetate (EA), acetone, 95% ethanol and water) in relation to its antioxidant capabilities via radical scavenging abilities of DPPH, hydrogen peroxide, superoxide anion, ferrous ion chelating capability and ferric reducing antioxidant power (FRAP); phytochemical analysis via total phenolic, flavonoid and flavanol contents; and inhibitory effects against xanthine oxidase (XO) and lipid peroxidation (LPO) using ferrous ion as ROS inducer on mice liver mitochondria. An insight on the effect of these factors to RSR capabilities could lead to a medicinal potential of this specie as suitable candidate for the development of a natural anti-gout and liver damage protective agent that have yet to be conducted.

MATERIALS AND METHODS

Chemicals and reagents

All reagents were of analytical grade. Catalase, horseradish peroxidase, xanthine oxidase were obtained from Calzyme laboratories Inc. Acetic acid was obtained from Kanto chemical Co., INC. Potassium phosphate (KH_2PO_4), sodium hydroxide (NaOH) and sodium phosphate (Na_2HPO_4, NaH_2PO_4) were obtained from J.T. Baker. Ferric chloride 6-hydrate ($FeCl_3 \cdot 6H_2O$) was obtained from Panreac. Hydrogen peroxide (H_2O_2) was obtained from Fluka. DPPH (2,2-diphenyl-1-picrylhydrazyl), 4-dimethylaminocinnamaldehyde (DMACA), allopurinol, aluminium chloride ($AlCl_3$), bovine serum albumin, dimethyl sulfoxide (DMSO), ethylene dinitrilotetraacetic acid (EDTA), epicatechin, ferrous chloride ($FeCl_2$), ferrozine, Folin-Ciocalteu's phenol reagent, gallic acid, methanol, phenazine methosulfate (PMS), nitro blue tetrazolium (NBT), , phenol red solution, rutin, superoxide dismutase (SOD), sodium acetate, 1,1,3,3-tetraethoxypropane (TEP), trolox, 2,4,6-tripyridyl-s-triazine (TPTZ), trifluoroacetic acid, 2-thiobarbi-turic acid (TBA), reduced form (β-NADH), xanthine and β-nicotinamide adenine dinucleotide were obtained from Sigma (St. Louis, MO).

Animals

ICR mice were obtained from BioLASCO Taiwan Co., Ltd. In accordance with the Laboratory Animal Ethics Committee of Taipei Medical University, the rats are used for this study was cared. In plastic cages mice were housed in the maintained temperature and with food and water, the animal house environment was kept on a 12-h light/12-h dark cycle. The experiments were conducted in accordance with the guidelines of the Experimental Animal Center of Taipei Medical University and the Chinese Society of Experimental Animal Science in Taiwan and the guidelines for the care and use of experimental animals were approved.

Extraction of plant and preparation of tested solution

RSR fresh stems, branches and leaves were harvested from Nantou County and Wulai District, New Taipei City

Table 1: Biological and pharmacological properties of compounds or crude extracts from RSR.[20]

Plant parts	Contents	Compound/ type of extract	Biological and Pharmacological properties
Leaves	Protocatechuic acid, p-coumaric acid, gallic acid, catechin, quercetin, methyl gallate, hydroxydammarenone, semialactone, moronic acid and betulonic acid	Gallic acid and methyl gallate/ ethanol and aqueous extracts	Antibacterial and antimicrobial
Stem		Hydroxydammar-enone, moronic and betulonic acid/ petroleum ether extract	Anticholesterol and antiviral
Branches		Semialactone	Anticholesterol
Fruits	Gallic acid, tannic acid and flavanoids	Methanol extract	Antidiarrhoeal
Gall	Gallotannins and gallic acid	Gallotannins and gallic acid /Ether, EtOAc, ethanol, methanol and aqueous extracts	Anticaries, antibacterial, antioxidant, anticholesterol and antidiabetic

in Taiwan (middle and northern Taiwan, respectively). Each voucher specimen were prepared and stocked at Department of Pharmacognosy, Taipei Medical University. Samples were cleaned and washed, then dried in the oven at 40°C for two days. Each dried sample of RSR (100 g) with the ratio 1:10 (Plant sample: fold solvents (*n*-hexane, EA, acetone, 95% ethanol and water)) was refluxed and extracted for twice. The combined crude extracts from each solvent were filtered, concentrated in vacuo (Eyela CCA-1111), then dried using lyophilizer (Eyela FDU-1200) and determined the percentage recovery. Each crude extract (20 mg) was dissolve in 1 mL DMSO as a stock solution and procedure is following the assays where the tested solution with PBS, methanol or distilled water (concentration of DMSO < 0.05%) is serially dilution method.

XO inhibitory capability of RSR extract

XO activity assay was performed according to Umamaheswari and Unno method with some modification. Briefly, each RSR extract was prepared at a series concentration where 50 µL of the tested solution was mixed with 35 µL of 1X PBS and solution containing XO (40 µL, 0.5 U/mL). After incubating at room temperature for 15 min, 50 µL of xanthine solution (100 µM) was added following to incubation for 30 min at room temperature before hydrogen chloride (25 µL, 1N) was added and mixed well. 295 nm absorbance was measured using ELISA reader (Synergy H4 Hybrid Reader). The DD-H_2O is blank group and the control group contains all reagents without the sample. Allopurinol was used as the positive control agent of XO inhibitor.[4,21] The XO inhibitory effect of each extract was calculated according to the following formula

$$\text{Percent Inhibition} = \frac{(A_{control} - A_{sample})}{(A_{control} - A_{blank})} \times 100$$

Each tested extract IC_{50} values was calculated by linear regression analysis.

Inhibitory capability of LPO on mice liver mitochondria

Preparation of mice liver mitochondria and protein content quantitation

The ICR mice (about 4 to 6 weeks) were sacrificed by carbon dioxide and the livers were removed immediately to perfuse with 0.1 M, pH 7.4 ice-cold PBS and homogenized using the homogenizer (Wheaton, Millville, NJ, USA). The homogenate is suspend in PBS, then centrifuged using 2000 rpm at 4°C for 10 min to separate. After re-centrifuge using 13000 rpm for 10 min at 4°C, The clear suspensions were finished to obtain mitochondrial fraction. Pipette mitochondrial suspensions of different concentrations into an eppendorf 1.5 mL volume and adjust the volume to 50 µL with PBS before adding the protein reagent and mixing with the vortex mixer. Absorbance was measured at 595 nm and a standard curve was generated from a plot of bovine serum albumin and the corresponding absorbance is used for protein in the mitochondrial solution quantitatively determination.

Inhibitory capability of each RSR extract on ferrous chloride induced LPO production in mice liver mitochondria

The LPO inhibitory activity was determined quantitatively by thiobarbituric acid reactive substances (TBARS) assay. A 500 µL reaction mixture solution (liver mitochondria 100 µL, PBS buffer 200 µL, $FeCl_2$ solution (4 mM) 100 µL and 100 µL of each extract or reference standard (trolox)) was incubated at 37°C for 1 hr and then centrifuged at 4000 rpm for 10 min. The reaction was added with H_3PO_4 375 µL, DD-water 200 µL and TBA 125 µL, then incubated at 90°C for 66 min. The termination of the reaction is following the addition of methanol-NaOH (the ratio 9.1: 0.9 v/v) 350 µL. MDA $(TBA)_2$ product 532 nm absorbance of formed was quantitatively measured against as a blank (DD H_2O only) and control (all reagents without the

sample). Data were recorded where LPO inhibition was calculated as mentioned earlier and each extract IC_{50} values were calculated by linear regression analysis.

LPO production of each RSR extract on mice liver mitochondria

A separate experiment for LPO production was measured using at least 2 or 3 times the concentration of IC_{50} values previously determined from ferrous chloride induced LPO inhibitory effect of the extracts where the same preparation without $FeCl_2$ addition and quantitative MDA $(TBA)_2$ product measurements were performed.

Antioxidant capability determination

Determination of antioxidant capabilities of each RSR extracts was evaluated using the previous method.[15]

DPPH free radical-scavenging capability

The DPPH radical scavenging activity of the extracts was performed where 100µL of different concentration of each RSR extract solutions in methanol and 50 mM DPPH 100 µL of solution in methanol were mixed, shaken and incubated in the dark at room temperature for 30 min. Absorbance measurements were carried out at 517 nm and methanol with DPPH solution as the standard and blank, respectively. The reaction mixture solution has lower absorbance, which indicated higher free radical-scavenging activity. Use the following formula to calculate the ability to scavenge DPPH free radicals

$$\text{Percentage scavenging} = \left(1 - \frac{\text{Asample}}{\text{Acontrol}}\right) \times 100$$

And each extract IC_{50} values are calculated by linear regression analysis.

Superoxide anion radical (O2˙-)-scavenging capability

Generation of O_2^{-} was done by dissolving O_2 which is ability to reduce NBT to purple formazan was measured in PMS-NADH coupling in a non-enzymatic system. Briefly, Add 936 µM β-NADH 250 µL and 300 µM NBT equal volume with 100 µL of a solution containing a series of concentrations (µg/mL) of extract. The reaction is added 120 µM PMS 250 µL to the mixture, then incubated at 37°C for 5 min. Absorbance at 560 nm was measured by control group that contains all the reagent where the amount of solution extracts was replaced with the same amount of DD H_2O and SOD (super oxidase) is the reference standard. Dissolve all the above reagents in freshly prepared in sodium phosphate buffer (pH 7.4). The superoxide anion radical (O_2^{-})-scavenging activity (%) was calculated as mentioned earlier and each extract IC_{50} values are calculated by linear regression analysis.

Hydrogen peroxide scavenging capability

Hydrogen peroxide scavenging ability of the RSR extracts were measured using 500 µL reaction mixture containing different concentration of each *RSR* extract solutions in methanol and 200 µL of 4 mM H_2O_2 solution, incubated at room temperature for 20 min followed by the addition of 300 µL red solution (phenol red 7.5 mM in 200 mM, HRPase 500 µg/mL and potassium phosphate buffer in pH 6.2). After another 10 min, then incubated at room temperature in an ice bath for 10 min to terminate the reaction afterwards its 610 nm absorbance was measured using ELISA reader. Catalase, 700 µL of ddH_2O with HRPase-phenol red solution (300 µL) and 500 µL of ddH_2O with 4 mM H_2O_2 (200 µL) and HRPase-phenol red solution (300 µL) were used as reference standard, blank and control group, respectively. The scavenging effect was calculated as mentioned earlier and each extract IC_{50} values are calculated by linear regression analysis.

Ferrous ion chelating capability

The each RSR extract ferrous ion chelating ability was monitored by 562 nm absorbance of the formed ferrous ion-ferrocene complex. The total volume of the reaction mixture containing each RSR extract or standard compound (EDTA), $FeCl_2$ (2 mM) and tetrahydrofuran (5 mM) was adjusted to 1 mL with methanol and then incubated at room temperature for 10 min. Lower absorbance indicates higher metal chelation activity. The ability of each RSR extract to chelate ferrous ions is expressed as

$$\text{Percent Chelating ability} = \left(1 - \frac{\text{Asample (562 nm)}}{\text{Acontrol (562 nm)}}\right) \times 100$$

Ferric reducing antioxidant power (FRAP)

FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 5 mM methanol TPTZ and 20 mM ferric chloride ($FeCl_3$) (10: 1: 1, v/v/v). Twenty-five microliter (25 µL) of extract solution (final concentration: 100 µg/mL) was mixed with 725 µL of the FRAP reagent for 4 min and absorbance was measured at 593 nm. Results were expressed as the trolox equivalent capacity values (µM).

Phytochemical analysis

Total phenolic content (TPC)

TPC of the extracts was estimated using Folin-Ciocalteu's reagent (10x diluted with DD water). Briefly, Folin-Ciocalteu's 500 µL was added to each extract 100 µL solution. After 30 sec, 7.5% Na_2CO_3 400 µL

was added and then incubated at 50°C for 30 min. Absorbance was measured at 765 nm against a blank. Gallic acid was used as reference standard.

Total flavonoid content

Total flavonoid content was estimated using aluminum chloride colorimetric method. Briefly, 500 µL of each RSR extract solutions were mixed with same volume 2% aluminum chloride (ratio 1:1) and then incubated at room temperature for 1 hr. The 430 nm absorbance was measured against a blank and rutin was used as a reference standard.

Total flavanol content

Each RSR extract solutions were mixed with the 4-dimethylaminocinnamaldehyde (DMACA). Absorbance was measured at 640 nm against a blank and epicatechin was used as a reference standard.

Statistical analysis

Data were presented as mean ± SD. All experiments were performed for triplicate. General linear model (Univariate) and Tukey's HSD post-hoc tests were applied to determine the influence of factors such as geographical source, aerial parts and extraction solvents used. The correlation is analysed by Pearson correlation where $p < 0.05$ was regarded as significantly different from the control group.

RESULTS

The percentage recovery of RSR extracts

Phytochemical constituents recovered in RSR depend on the nature of extracting solvents used. Solvents with different polarities (*n*-hexane, ethyl acetate, acetone, 95% ethanol, water) were used and such variations affect the percentage recovery as well as bioactivities of the extracts due to complex structure and nature of these phytoconstituents. According to Table 1, RSR methanol and aqueous extracts showed that it contained phenolic compounds. The results of Table 2 showed that the ML ethanol and aqueous after *n*-hexane extract have larger yield more than other parts extracts. And non-polar *n*-hexane extract showed the poor extraction yield.

XO inhibition, LPO production and LPO inhibitory capability

Xanthine oxidase (XO) is plays the important role in human purine nucleotide metabolism. Its main role is to catalyze the oxidation of hypoxanthine to xanthine and xanthine to uric acid.[22] Over production and/or under excretion of XO caused ROS production and increased uric acid generation leading to hyperuricemia such as gout. Treatment for gout patient includes XO inhibitor that blocks the production of uric acid. Allopurinol, benzbromarone, NSADS and colchicine are commonly used but have been reported with adverse side effects like hepatotoxicity that relates to LPO production since it can be damaged liver tissues to pathological conditions.

Table 2: The yield (%) of each RSR extract.

RSR[*a]	Yield (%) [*b]				
	n-hexane	EA	Acetone	Ethanol	Water
MS	0.70	1.24	1.66	0.14	3.77
MB	1.90	5.45	9.10	9.90	12.70
ML	4.95	9.53	6.40	16.40	21.80
NS	0.63	1.94	1.10	2.84	2.33
NB	0.90	3.00	2.25	7.73	9.40
NL	3.60	8.54	6.87	16.62	18.20

[*a] Geographical source and aerial part-
M: Middle Taiwan; N: Northern Taiwan
S: Stem; B: Branch; L: Leaf
[*b]: The yield was calculated by the extract weight/dried material (RSR) weight ×100%

In this study, the capability of RSR extracts to inhibit XO and LPO production were determined. Based on the results of Table 3 showed that ML-*n*-hexane, MS-*n*-hexane, NS-ethanol extracts are more potential to develop as XO inhibitor (IC_{50}: 16.74±0.74, 26.53±0.54 and 26.80±0.39 µg/mL, respectively), in comparison to the positive control (allopurinol) (IC_{50}: 0.57±0.02 µg/mL). On the other hand, NS-acetone, NL-water and NL-EA extract exerted the potential inhibitory effects on ferrous ion-induced LPO (IC_{50}: 8.40±0.35, 9.42±1.11, 10.43±0.46 µg/mL, respectively) as compared to positive control (trolox) (IC_{50}: 9.21±0.32 µg/mL). All extracts tested did not induced LPO production even with the concentration of over double doses of IC_{50} was applied. Discussion of potential phytochemical of RSR ML-*n*-hexane, MS-*n*-hexane and NS-ethanol, leaves and stem extracts may contained phenolic components as its target compound to be the XO inhibitor and showed the inhibitory effect on iron-induced LPO.[20]

Antioxidant capabilities and phytochemical analysis

An imbalance between ROS and antioxidant enzyme in the body will react with protein, membrane and DNA. Toxic LPO products, carcinogenic MDA,[23] and XO production contributed to ROS production. Recently, the antioxidant treatment has been seriously considered as an effective strategy because antioxidants can decrease LPO reaction and scavenge free radical to counteraction the ROS damage.[24] Therefore, in this study, antioxidant capabilities and phytochemical analysis of RSR were evaluated. All antioxidant capabilities and phytochemical

Table 3: The IC_{50} (μg/mL) of XO and LPO inhibitory capabilities of RSR extracts.

RSR*[a]	Extract solvent	Xanthine oxidase	LPO
MS	*n*-hexane	26.53±0.54	55.24±0.63
	EA	43.87±0.11	10.78±0.74
	Acetone	85.45±2.91	13.17±1.11
	Ethanol	148.10±0.38	30.26±0.36
	Water	119.44±9.83	21.34±0.29
MB	*n*-hexane	>300	29.67±1.77
	EA	>300	51.32±2.34
	Acetone	190.14±1.84	88.98±7.42
	Ethanol	78.81±1.51	66.27±2.03
	Water	>500	40.52±1.43
ML	*n*-hexane	16.74±0.74	72.92±2.57
	EA	>300	13.52±3.19
	Acetone	>300	28.68±0.76
	Ethanol	>300	23.94±0.86
	Water	>300	14.52±0.48
NS	*n*-hexane	>300	19.41±1.23
	EA	66.72±7.40	21.36±1.68
	Acetone	38.18±2.71	8.40±0.35
	Ethanol	26.80±0.39	25.86±0.30
	Water	101.69±13.60	31.83±0.71
NB	*n*-hexane	129.31±2.32	48.36±2.30
	EA	76.31±5.94	40.16±1.08
	Acetone	177.31±7.72	22.66±1.88
	Ethanol	72.20±0.47	43.35±3.12
	Water	74.35±2.73	54.72±3.37
NL	*n*-hexane	>120	43.36±1.50
	EA	>200	10.43±0.46
	Acetone	128.62±3.16	37.22±0.45
	Ethanol	>300	22.35±0.89
	Water	>300	9.42±1.11
Positive control	--	(Allopurinol) 0.57±0.02	(Trolox) 2.52±0.37

*[a] Geographical source and aerial part-
M: Middle Taiwan; N: Northern Taiwan
S: Stem; B: Branch; L: Leaf

analysis of each RSR extract were shown in Table 4 and Table 5, respectively. Results showed that MS-EA extract gave the highest superoxide anion radical scavenging (IC_{50}: 53.48±0.75 μg/mL), FRAP (equivalent trolox 116.40±0.34 μM) and DPPH scavenging activity (IC_{50}: 1.53±0.08 μg/mL) while the best hydrogen peroxide-scavenging capability was observed in MS-acetone extract. Additionally, phytochemical analysis showed that higher total phenolic content was in MS, especially in MS acetone and EA extracts (455.17± 9.29 and 425.64±6.09 μg Gallic acid/mg), higher total flavonoid (ML-EA extract: 73.24±5.04 μg Rutin/mg) and flavanol (NL-EA extract: 110.65±7.48 μg epicatechin/mg) contents were mainly in leaf extracts. Phenolic compounds have been found from RSR stem extract.[20] The phenolic compounds have been showed various biological activities. The literature also showed that ferulic acid and gallic acid related phenolic compounds were found against XO and COX-2 for anti-inflammatory activity.[25]

DISCUSSION

Correlation between XO and LPO inhibition, antioxidant capabilities and phytochemical contents

Cell damages due to ROS are related to LPO productions and the antioxidant capabilities of plant extract that can inhibit the LPO production could lead to overall protection against cell damages, aging and necrosis. In this study, LPO inhibition with DPPH radical scavenging (r= 0.507, p=0.004) and FRAP (r= 0.521, p=0.003) was median relation and showed a relationship with polyphenol content (r= 0.496, p=0.005), flavonoid content (r= 0.461, p=0.010) and flavanol content (r= 0.421, p=0.040). These possibly explain that the antioxidant and radical scavenging ability of the extract are effective strategy for liver protection where ROS is involved in the main mechanisms of liver injury. Furthermore, FRAP exerted high correlation with DPPH scavenging capability (r= 0.702, p<0.001) and total phenolic content (r= 0.833, p<0.001) suggesting a strong relationship of phenolic compounds present in the extract with its antioxidant activities. The flavonoid content with flavanol content showed high linear correlation (r= 0.838, p<0.001) and RSR leaf polar extracts with higher flavonoid and flavanol content which possibly suggest that flavonoids and flavanols are largely distributed in the highly exposed plant part to sunlight due to its high-sunlight response.[26] The XO inhibitory effect showed low correlation with other activities and non-polar extracts exerted higher XO inhibition, albeit some reports found out that phytochemical constituent of some plant extracts in solvents with lower polarity than water exhibit significant XO inhibitions as compared to standard.[22,27,28]

The influence of geographical sources, aerial parts and extraction solvents on the XO and LPO inhibitory capabilities, antioxidant capabilities and RSR extracts phytochemical content analysis

The factor influence that is including variations in geographical source, aerial parts and extraction solvents of RSR extracts, except the hydrogen peroxide scavenging activity was summarized (Figure 1). The solvents polarities play a main factor in DPPH (water, EA and ethanol extracts) and superoxide anion (acetone and water extracts) scavenging capabilities, XO inhibitory effects (*n*-hexane and EA extracts) and FRAP (water extract) since it relate to the nature of extracts in terms

Table 4: Antioxidant capabilities of RSR extracts.

RSR[*a]	Extract solvent	DPPH	Hydrogen peroxide	Superoxide anion	Fe^{2+} chelating capability	FRAP
		IC_{50} (μg/mL)			Scavenging ratio (%)	Equiv. trolox (μM) [*b]
MS	*n*-hexane	56.25±1.13	>300	226.56±2.07	0	6.00±0.14
	EA	1.53±0.08	264.25±4.75	53.48±0.75	0	116.40±0.34
	Acetone	1.65±0.16	208.03±1.87	63.47±2.94	0	114.81±0.59
	Ethanol	8.03±0.62	>300	80.89±2.93	0	56.40±0.40
	Water	5.85±0.26	>300	58.48±2.45	9.20±2.43	110.52±1.34
MB	*n*-hexane	109.12±17.67	>300	141.09±3.49	0	3.68±0.09
	EA	23.21±5.39	>300	>100	0	17.41±0.39
	Acetone	78.68±5.38	>300	>100	0	7.37±0.14
	Ethanol	24.04±0.60	>300	>100	0	30.67±0.28
	Water	6.86±0.78	>300	199.52±4.28	0	53.74±0.41
ML	*n*-hexane	113.46±6.56	>300	>100	0	7.11±0.09
	EA	17.26±0.85	>300	144.53±2.00	4.92±0.92	66.26±1.34
	Acetone	12.97±0.66	>300	>100	0	35.62±0.61
	Ethanol	13.32±0.89	>300	>100	0.78±0.19	61.94±1.03
	Water	5.15±0.41	504.47±3.70	53.79±0.31	0	110.74±0.25
NS	*n*-hexane	46.75±2.73	>300	>100	0	9.69±0.09
	EA	12.60±0.59	>300	>100	0	29.29±0.17
	Acetone	22.87±1.61	>300	>100	0	26.25±0.01
	Ethanol	7.82±0.31	>300	>100	0	46.56±0.42
	Water	7.75±0.42	>300	107.20±0.26	5.96±0.74	75.93±0.30
NB	*n*-hexane	135.53±2.90	>300	>100	0	5.29±0.05
	EA	15.19±0.83	>300	223.29±1.95	0	42.35±0.77
	Acetone	51.04±3.05	>300	>100	0	15.58±0.16
	Ethanol	23.76±0.05	>300	>100	0	41.97±0.15
	Water	7.72±0.47	>300	54.34±1.49	8.23±0.34	91.31±1.50
NL	*n*-hexane	90.70±8.17	>300	>100	0	10.56±0.05
	EA	20.44±0.99	>300	100.62±1.13	6.93±1.84	52.99±0.36
	Acetone	21.87±1.64	>300	>100	0	34.35±0.25
	Ethanol	15.58±0.48	>300	190.11±1.14	7.12±0.34	49.67±0.16
	Water	6.07±0.50	>300	125.82±1.44	12.18±0.92	111.34±1.64
Positive control	--	(Gallic acid) 0.86±0.02	(Catalase) 1.51±0.01 U/mL	(Superoxidase) 84.62±3.19 U/mL	--	--

*0: no Fe^{2+} chelating capability was detected

[*a] Geographical source and aerial part-
M: Middle Taiwan; N: Northern Taiwan
S: Stem; B: Branch; L: Leaf

[*b]: FRAP of each extract was showed as trolox equivalent (μM) at the concentration of 100 μg extract per milliliter

Table 5: Phytochemical analysis of RSR extracts.

RSR*[a]	Extract solvent	Total phenolic µg Gallic acid/ mg*[b]	Total flavonoid µg Rutin/ mg*[b]	Total flavanol µg epicatechin /mg*[b]
MS	*n*-hexane	81.98±2.07	0.91±0.66	0
	EA	425.64±6.09	18.15±1.47	3.72 ±0.66
	Acetone	455.17± 9.29	30.94±2.66	4.77 ±0.30
	Ethanol	238.98±5.64	9.90±1.20	1.90±0.05
	Water	207.11±2.94	7.78±0.37	10.92±0.05
MB	*n*-hexane	9.37±3.57	5.14±0.97	0
	EA	24.18±0.50	10.22±1.50	11.16±0.49
	Acetone	22.24±5.49	8.52±0.84	3.00±0.28
	Ethanol	52.79±0.82	8.21±0.37	11.29±0.49
	Water	88.56±2.07	6.94±0.18	17.89±0.20
ML	*n*-hexane	8.63±1.54	11.59±1.80	0
	EA	101.66±1.72	73.24±5.04	78.36±2.00
	Acetone	65.56±1.88	34.01±1.80	42.05±1.64
	Ethanol	140.66±2.30	54.95±4.67	60.38±2.05
	Water	328.19±9.92	43.21±1.50	86.50±1.35
NS	*n*-hexane	18.12±8.53	2.18±0.48	0
	EA	117.81±1.45	5.46±0.18	9.60±0.20
	Acetone	53.47±1.20	5.67±0.32	7.08±0.29
	Ethanol	165.45±1.90	5.99±1.02	14.54±1.04
	Water	178.14±3.10	3.66±0.00	23.96±0.19
NB	*n*-hexane	15.75±0.33	5.67±2.40	0
	EA	127.33±1.84	12.65±1.28	23.29±0.63
	Acetone	30.61±1.61	10.00±1.57	12.20±0.75
	Ethanol	52.46±0.50	9.79±0.00	11.88±0.68
	Water	141.32±8.46	6.20±0.92	11.59±0.00
NL	*n*-hexane	18.89±0.24	9.37±1.98	0
	EA	174.84±2.97	51.78±5.32	110.65±7.48
	Acetone	62.77±13.02	29.89±4.12	40.95±2.59
	Ethanol	102.52±0.68	52.41±4.62	43.29±0.61
	Water	123.64±5.09	45.85±1.60	64.00±0.00

*o: Total flavanol content cannot be detected

*[a] Geographical source and aerial part-
M: Middle Taiwan; N: Northern Taiwan
S: Stem; B: Branch; L: Leaf

*[b]: all expressed in terms of standard equivalent (µg of equivalent standard/ mg of extract)

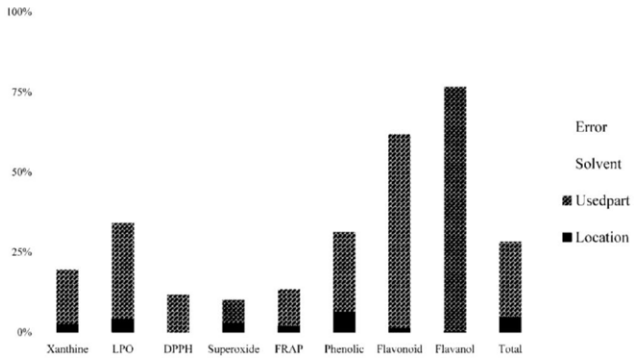

Figure 1: The influence factors on the XO and LPO inhibitory capabilities, antioxidant capabilities and phytochemical analysis.

*Error: error term, unable to be explained by these three factors in the linear relationship.

of the phytochemical constituents that dissolve in it. Variations in aerial parts are more influential in flavonoid and flavanol contents (60.49% and 76.24%), where leaf extracts exhibit the maximum possibly due to its direct exposure to sunlight. Likewise, extracts from aerial part of RSR used in the study significantly affected LPO inhibition (stem and leaf) and total phenolic content (stem extracts higher) more than other factors. In summary, variation in extraction solvents (27.37%) and aerial parts (23.43%) mainly influenced the RSR activities, while variation in geographical source (4.80%) has no significant impact on these activities suggesting that Middle and Northern Taiwan environment share the same primary ecological and climate factors influencing the active substance production of RSR including the annual average precipitation, temperature, sunshine duration, soil pH, soil organic matter and rapidly available potassium in the soil that well described as being able to influence the production of metabolites.[29,30]

CONCLUSION

The leaf and stem of RSR are potential bio-source for developing anti-gout agent due to significant antioxidant activities leading to inhibitory effects against XO and LPO productions with all extracts exhibited liver damage protection. Results from Pearson correlation and general linear model (Univariate) suggest that polarities of different extraction solvents and aerial parts of RSR are mainly the influential factors to the observed activities. This study could give an insight about RSR as a potential candidate plant source to develop a natural anti-gout agent with liver damage protective ability that have yet to be conducted.

Author Contributions

Conceptualization, P.-W.T. and L.-L.Y.; methodology, formal analysis and data curation, P.-C.L., B.-C.W., M.-S.W. and L.-L.Y.; writing—original draft preparation, P.-W.T., A.-H.L., S.-J.S. and P.-C.L.; supervision, B.-C.W. and M.-S.W. All authors read and approved the final manuscript. P.-W.T. and P.-C.L. contributed equally to this work.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

ABBREVIATIONS

RSR: *Rhus semialata* var. *roxburghiana;* **XO:** Xanthine oxidase; **FRAP:** Ferrous ion chelating capability; **TPC:** Total phenolic content; **MS:** Middle Taiwan RSR stem; **MB:** Middle Taiwan RSR branch; **ML:** Middle Taiwan RSR leaf; **NS:** Northern Taiwan stem; **NB:** Northern Taiwan RSR branch; **NL:** Northern Taiwan RSR leaf.

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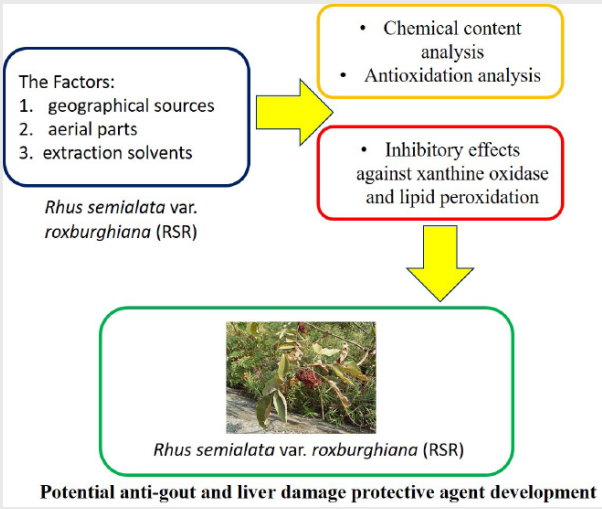
PICTORIAL ABSTRACT



SUMMARY

Gout, a disease characterized by recurrent inflammatory for urate deposit from overactive xanthine oxidase, is common among Taiwanese. Rhus semialata var. roxburghiana (RSR) grows wildly in Taiwan and used by aboriginal Taiwanese as traditional medicine for gout and hepatitis. The leaf and stem of RSR are potential bio-source for developing anti-gout agent due to significant antioxidant activities leading to inhibitory effects against XO and LPO productions with all extracts exhibited liver damage protection. Results from Pearson correlation and general linear model (Univariate) suggest that polarities of different extraction solvents and aerial parts of RSR are mainly the influential factors to the observed activities. Overall, RSR could lead to a medicinal potential as a suitable candidate for the development of a natural anti-gout and liver damage protective agent that have yet to be conducted.

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