

## Polyphenol-enriched *Sophora japonica* L. and *Rosa rugosa* Thunb. composite solid beverage: antioxidant and lipid-lowering efficacy with stability assessment

Jianzhuo Du, Yunxiao Xia, Pingyu Ge, Xu Zhao \*

Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang 110016, China

**Abstract** In the context of a surging demand for functional foods, this study utilized *Sophora japonica* L. (SL) and *Rosa rugosa* Thunb. (RT), which are rich in polyphenols (with flavonoids as the core subclass). High-purity extracts (SLE and RTE) were obtained through ethanol reflux extraction and macroporous resin purification, and then formulated with maltodextrin and erythritol to prepare a composite solid beverage. This beverage exhibited excellent antioxidant capabilities. At a concentration of 1 mg/mL, the scavenging rates of DPPH, ABTS, and hydroxyl radicals reached 82.4%, 94.6%, and 49.2%, respectively. Network pharmacology indicated that quercetin and  $\beta$ -sitosterol could modulate lipid metabolism pathways. Moreover, the beverage showed potential for lipid-lowering. Its cholate adsorption capacity was  $589.4 \pm 2.9$  mg/g at pH 7.0, and the  $IC_{50}$  value for pancreatic lipase inhibition was 32.55 mg/mL. However, a 60-day storage stability test revealed that the moisture content approached 5%, likely due to polyphenol-flavonoid reactivity, extending dissolution time to 30.88 s. These changes were attributed to polyphenols (with flavonoids as the core active subclass, and non-flavonoids such as phenolic acids as auxiliary), resulting in color alterations and reduced solubility. This study confirmed the dual functions of the SL-RT beverage in antioxidant and lipid-lowering aspects. Nevertheless, it also pointed out the need to optimize the formula and process to enhance stability, providing an important basis for the development of stable functional beverages.

**Keywords:** *Sophora japonica* L.; *Rosa rugosa* Thunb.; polyphenols; flavonoids; antioxidant activity; lipid metabolism regulation; network pharmacology

### 1 Introduction

In the context of escalating global demand for functional foods to address metabolic disorders, polyphenols (with flavonoids as a key bioactive subclass) have gained prominence as critical bioactive agents, owing to their dual capacity for antioxidant defense and lipid metabolism modulation<sup>[1-3]</sup>. These phytochemicals, ubiquitously present in edible plants, are renowned for mitigating oxidative stress

and regulating lipid absorption pathways, thereby emerging as cornerstone components in dietary strategies targeting metabolic syndromes<sup>[4,5]</sup>.

*Sophora japonica* L. (SL) and *Rosa rugosa* Thunb. (RT), two traditional medicinal-edible botanicals, are particularly enriched medicinal-edible botanicals, are particularly enriched with polyphenolic constituents (e.g., phenolic acids, proanthocyanidins) and flavonoid derivatives (e.g., rutin, quercetin), which exhibit synergistic contributions to their health-promoting efficacy<sup>[6-8]</sup>. Proanthocyanidins typically exist in plant tissues in the forms of monomers,

\* Corresponding author: Xu Zhao (zhaoxu\_1010@163.com).

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oligomers, and polymers, with catechin or epicatechin as their basic structural units <sup>[9]</sup>. Although there are currently no direct reports on proanthocyanidin monomers in SL and RT, it can be inferred from the widespread existence characteristics of proanthocyanidins and research experiences on other plants (e.g., aronia, whose proanthocyanidins contain 0.78% monomers) that proanthocyanidin monomers are highly likely to exist in the raw materials of this beverage. This may be one of the contributing forms to the total proanthocyanidin content ( $9.23 \pm 1.98$  mg/g). Notably, rutin from SL and quercetin from RT demonstrate exceptional free radical scavenging properties, while their structural diversity facilitates interactions with lipid-metabolizing enzymes (e.g., pancreatic lipase) and bile acid enterohepatic circulation, highlighting their therapeutic potential in hyperlipidemia management <sup>[10,11]</sup>.

Given the bioactivity of SL and RT extracts, solid beverage formulations were chosen to enhance polyphenol-flavonoid delivery, present a viable platform to harness the polyphenol-flavonoid synergy of SL and RT extracts. Prior studies indicate that polyphenol-flavonoid complexes exhibit enhanced antioxidant activity compared to isolated compounds, mediated through redox cycling, hydrogen atom transfer, and metal chelation mechanisms <sup>[12, 13]</sup>. Furthermore, these compounds impede lipid digestion via pancreatic lipase inhibition and cholesterol/bile acid adsorption, effectively reducing intestinal lipid uptake <sup>[14, 15]</sup>. Despite these advancements, research on SL-RT composite systems remains sparse, particularly regarding the interplay between their polyphenolic and flavonoid profiles in conferring dual antioxidant and hypolipidemic effects. Additionally, the stability of such formulations during storage—a critical determinant of commercial feasibility—has not been comprehensively investigated, leaving gaps in mechanistic elucidation and practical implementation.

To address these limitations, this study developed an SL-RT composite solid beverage optimized for polyphenol and flavonoid retention. High-purity extracts (SLE and RTE) were prepared using ethanol reflux extraction coupled with macroporous resin

purification, ensuring maximal bioactive preservation. The synergistic effects of these extracts were systematically evaluated through *in vitro* antioxidant assays (DPPH, ABTS, and hydroxyl radical scavenging) and lipid metabolism regulation studies (cholesterol adsorption, pancreatic lipase inhibition, and network pharmacology-driven pathway analysis). Stability parameters, including moisture content, dissolution kinetics, and colorimetric profiles, were monitored over 60 days to delineate degradation mechanisms. By elucidating the roles of polyphenols and flavonoids in SL-RT synergy and addressing formulation challenges, this work provides a framework for developing stable, functionally enhanced beverages tailored for metabolic health interventions.

## 2 Materials and methods

### 2.1 Acquisition of main active components

The main chemical constituents of SL and RT were searched by TCMSP Pharmacologic database and analysis platform (<https://old.tcmsp-e.com/>).

### 2.2 Materials

The acacia rice powder and rosehip powder used in this study were obtained from Shanghai Jinliang Food Technology Co. Maltodextrin, erythritol and edible ethanol were food grade. Olive oil, egg and soybean oil were purchased from supermarkets. Rutin, gallic acid and proanthocyanidins were analytical quality. DPPH free radical assay kit, total antioxidant activity assay kit and OH<sup>-</sup> free radical assay kit were obtained from Nanjing Jiancheng Bioengineering Institute, and ABTS free radical assay kit was obtained from Beijing Solarbio Science & Technology Co., Ltd.

### 2.3 Extraction of active ingredients of SL and RT

The crude powder of SL was weighed precisely, and 60% ethanol solution was prepared, the material-liquid ratio was 1:15 g/mL, and the extract was refluxed in a water bath with heat for 1.25 h. The

filtrate was purified by a macroporous resin, and the samples of SL extract (SLE) were prepared.

The crude powder of RT was weighed precisely, and 50% ethanol solution was prepared, the material-liquid ratio was 1:13 g/mL, and the extract was refluxed in a water bath with heat for 1.5 h. The filtrate was purified by a macroporous resin, and the samples of RT extract (RTE) were prepared.

## 2.4 Development of SL and RT solid beverage

SLE, RTE, maltodextrin, and erythritol were weighed according to the recipe amount and set aside. The raw materials, excipients and additives were mixed well and a wetting agent was added to make a soft material, which was extruded through a 14-mesh screen to make granules. After drying and finishing, the solid beverage of SL-RT is produced.

## 2.5 Antioxidant activity

### 2.5.1 Total phenol content, total flavonoid content and proanthocyanidin content

Total phenolic content was determined using Folin-Phenol reagent according to the procedure described by Nowak and Gośliński<sup>[16]</sup>. Total flavonoid content was determined according to the Chinese local standard (DB 34/T 2743-2016). Proanthocyanidin content was determined as follows. 1 mL of SL-RT solid beverage solution was put into a 10 mL volumetric flask, 6 mL of 40 mg/mL vanillin-methanol was added and shaken well, and 3 mL of concentrated hydrochloric acid was added and shaken well. The reaction was carried out in a water bath at 30 °C for 20 min, and the absorbance was measured at 500 nm.

The reaction was carried out in a water bath at 30 °C for 20 min. The absorbance was measured at a wavelength of 500 nm and zeroed with a blank solution.

### 2.5.2 DPPH free radical, total antioxidant activity, OH<sup>-</sup> free radical, and ABTS free radical scavenging

Samples were prepared under optimal process

conditions and total antioxidant capacity was assessed using DPPH free radical assay kit, total antioxidant activity assay kit, OH<sup>-</sup> free radical assay kit, and ABTS free radical assay kit. The experiments were manipulated and results were obtained according to the instructions.

## 2.6 Lipid metabolism

### 2.6.1 Network pharmacology

Using the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) (<https://old.tcmsp-e.com/>), the UniProt database (<https://www.uniprot.org/>), the GeneCard database (<http://www.genecards.org/>), the OMIM database (<http://omim.org/>), the Venny 2.1 website (<https://bioinfogp.cnb.csic.es/tools/venny/>), the String database (<https://string-db.org/>), the Metascape database (<https://metascape.org/>) and Cytoscape 3.9.1 software for analysis and mapping.

### 2.6.2 Cholate adsorption activity

Cholate adsorption activity was determined by the modified Zheng et al<sup>[17]</sup> method. The inhibition of pancreatic lipase was referred to some modifications in the literature<sup>[18, 19]</sup>. The detailed experimental methods were as follows: a mixture of PBS (pH = 7.4) and 1 mL of 4% polyvinyl alcohol olive oil (volume ratio 3:1) was preheated for 10 min in a thermostatic shaker at 37 °C. After preheating, 1 mL of the sample was added and left at 37 °C for 10 min, followed by 1 mL of 100 g/L pancreatic lipase and placed in a thermostatic shaker at 37 °C for 15 min. The reaction was terminated by the addition of 95% anhydrous ethanol, followed by the addition of 3 drops of phenolphthalein indicator and titration with NaOH solution until slightly red. After measuring the enzyme activity, the inhibition rate was calculated.

$$\text{Enzyme activity} = 1000 \times \frac{(V_1 - V_2 - V_3) \times c}{t \times w} \times 100\%$$

$$\text{Inhibition rate} = \frac{(U_1 - U_2)}{U_1} \times 100\%$$

Where  $V_1$  is the volume of NaOH solution consumed by the sample group,  $V_2$  is the volume of NaOH solution consumed by the blank group,  $V_3$  is the volume of NaOH solution consumed by the control group,  $c$  is the concentration of NaOH standard solution,  $t$  is the reaction time after the addition of pancreatic lipase,  $w$  is the amount of pancreatic lipase added,  $U_1$  is the activity of pancreatic lipase before inhibition, and  $U_2$  is the activity of pancreatic lipase after inhibition.

The cholesterol adsorption activity was the same process described by Chu et al [20] and Xu et al [21]. The adsorption experiments were performed at pH 2.0 and pH 7.0 to simulate the pH environment of the stomach and intestine, respectively.

The soybean oil adsorption capacity was determined by the method of Nsor-Atindana et al [22]. The amount of oil-binding capacity to the SL-RT solid beverage is expressed in (g/g).

## 2.7 Shelf life

Store SL-RT solid beverage in a sealed bag away from light and observe its indicators regularly at room temperature.

### 2.7.1 Moisture content

The moisture content was determined by direct drying method and determined according to the Chinese standard GB 5009.3-2016.

### 2.7.2 Color difference values

The SL-RT solid beverage was placed on a colorimeter with the measuring surface tidied and the values in the colorimeter were expressed in terms of  $L^*$ ,  $a^*$ , and  $b^*$ .  $L^*$  indicates brightness,  $L^* = 0$  indicates black and  $L^* = 100$  indicates white.  $a^*$  indicates red-greenness, a larger value of  $a^*$  indicates darker red,  $a^* < 0$  indicates greenness, a smaller value of  $a^*$  indicates greater greenness.  $b^*$  indicates yellow-blueness,  $b^* > 0$  indicates the degree of yellow, a larger  $b^*$  value indicates a deeper degree of yellow,

$b^* < 0$  indicates the degree of blue, a smaller  $b^*$  value indicates a greater degree of blue.

### 2.7.3 pH value

Pour the SL-RT solid beverage into a beaker, insert the electrode of the pH meter into the filtrate, and read the pH value of the sample when the reading is stable.

## 2.8 Statistical analysis

Statistical analysis was performed using a one-way analysis of variance and regression coefficient analysis, with results expressed as mean  $\pm$  standard deviation, and  $P < 0.05$  was considered statistically significant.

## 3 Results and discussion

### 3.1 Acquisition of main active components

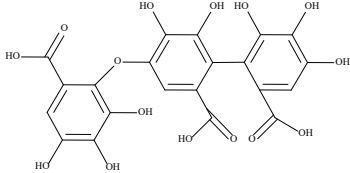
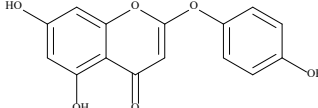
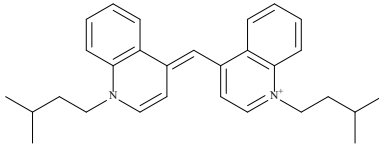
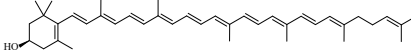
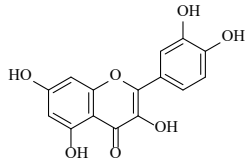
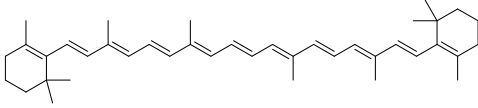
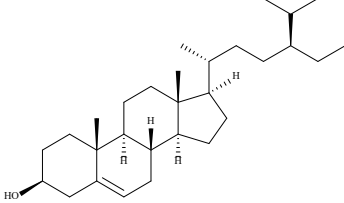
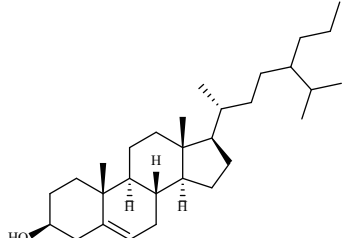
Among the 12 active components screened via the TCMSP database (Table 1), polyphenol-related components include flavonoids, such as quercetin (flavonol), Rugosin D (flavonoid glycoside); proanthocyanidins (flavanol subclass of flavonoids). Specifically, quercetin is classified as a flavonoid (a subclass of polyphenols) and *Rugosin D* as a phenolic derivative. Additionally, experimental determinations reveal that the beverage contains total phenols ( $36.41 \pm 2.01$  mg/g, including flavonoids  $11.76 \pm 0.05$  mg/g and non-flavonoid phenolic acids, etc.), among which flavonoids include flavonols (such as quercetin) and flavanols (proanthocyanidins  $9.23 \pm 1.98$  mg/g) — these collectively form the polyphenolic basis of the beverage.

Notably, quercetin, as a typical flavonol (a secondary subclass of flavonoids, with a 3-hydroxy flavone skeleton), is present in both raw materials (OB = 46.43%, DL = 0.28). Its structural characteristics endow it with potential for free radical scavenging and lipid metabolism regulation [23], making it a likely key contributor to the beverage's functionality. The

DPPH scavenging rate of the beverage (82.4%) in this study may be directly associated with its contribution. Moreover, the determined total proanthocyanidin content suggests the presence of proanthocyanidins with varying degrees of polymerization in the

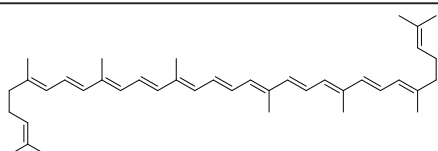
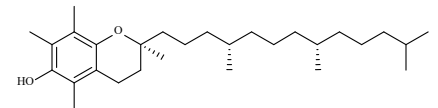
beverage. Given that proanthocyanidins are formed through the gradual polymerization of monomers, it is reasonable to speculate that proanthocyanidin monomers are highly likely to exist in the beverage.

**Table 1 Information for the candidate bioactive compounds of RT**

MOL ID	Compound	MF	OB/%	DL	Structure
MOL010738	RugosinD_qt	C <sub>75</sub> H <sub>54</sub> O <sub>48</sub>	57.29	0.67	
MOL008046	Demethoxycapillarisin	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	52.33	0.25	
MOL006209	cyanin	C <sub>29</sub> H <sub>35</sub> N <sub>2</sub>	47.42	0.76	
MOL010736	rubixanthin	C <sub>40</sub> H <sub>56</sub> O	47.26	0.53	
MOL000098	quercetin	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	46.43	0.28	
MOL002773	$\beta$ -carotene	C <sub>40</sub> H <sub>56</sub>	37.18	0.58	
MOL000358	$\beta$ -sitosterol	C <sub>30</sub> H <sub>52</sub> O	36.91	0.75	
MOL000359	sitosterol	C <sub>29</sub> H <sub>50</sub> O	36.91	0.75	

(to be continued)

**Continued Table 1**

MOL ID	Compound	MF	OB/%	DL	Structure
MOL010267	LYC	C <sub>40</sub> H <sub>56</sub>	32.57	0.51	
MOL007180	vitamin-e	C <sub>66</sub> H <sub>106</sub> CaO <sub>10</sub>	32.29	0.7	

Note: OB: Oral bioavailability; DL: Drug-likeness, thresholds: OB ≥ 30%, DL ≥ 0.18 for screening.

### 3.2 Antioxidant activity

Polyphenols detected in this study are mainly reflected by three detection indicators, and their chemical classification hierarchy is: total phenols (the broadest category, covering flavonoids and non-flavonoids such as phenolic acids), total flavonoids (a subclass of polyphenols, belonging to the C6-C3-C6 skeleton flavonoid category), proanthocyanidins (a subclass of total flavonoids specifically polymers of flavanols). Among them, total flavonoids and proanthocyanidins are the main existing forms of polyphenolic components in the beverage, which is consistent with the characteristic of SL and RT being rich in flavonoids. The higher the content of natural functional ingredients, the more beneficial they are to health [24, 25]. The solid beverage prepared in this study also detected different levels of active ingredients due to the addition of SL and RT, which gave it some nutritional value and antioxidant activity.

DPPH is a synthetic radical, insoluble in water, soluble in ethanol and methanol solution, with a dark purple color and a certain stability. The absorbance level is proportional to the antioxidant activity and thus the antioxidant activity of the sample can be judged [26]. As can be seen from Fig. 1A, the scavenging of DPPH free radical increased with increasing concentration of the SL-RT solid beverage, and the DPPH scavenging rate was 82.37% when the solution concentration of the solid beverage was 1 mg/mL. Although the antioxidant activity of the solid beverage was not as high as that of Vc, the solid

beverage also showed some antioxidant activity.

There are many antioxidant substances in the organism that can reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>, which can form stable complexes with pheophorbides, and the level of their antioxidant activity can be measured by colorimetry. As shown in Fig. 1B, the total antioxidant activity of the SL-RT solid beverage increased with the increasing concentration of the solution, which was 2.05 U/mL at a concentration of 1 mg/mL and 26.69 U/mL at a concentration of 10 mg/mL. The antioxidant activity of the solid beverage prepared in this study was good.

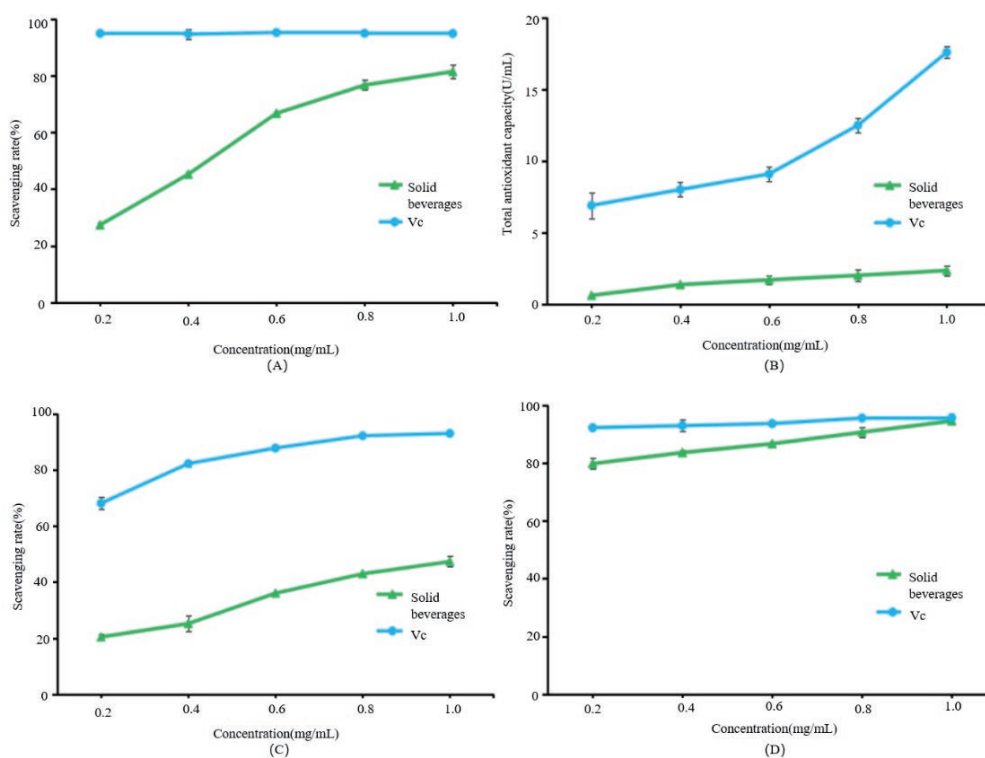
The amount of H<sub>2</sub>O<sub>2</sub> is proportional to the amount of OH<sup>-</sup> produced by the Fenton reaction, and the free radicals produced by the Fenton reaction react with Griess reagent to produce a red substance, the color of which is proportional to the amount of OH<sup>-</sup> [27]. As can be seen from Fig. 1C, the scavenging rate of OH<sup>-</sup> increased with the increasing concentration of SL-RT solid beverage solution, and at a concentration of 1.0 mg/mL, the scavenging rate of OH<sup>-</sup> reached 49.2 ± 1.20% and 93.3 ± 1.55% for solid beverage and Vc respectively. The solid beverage of SL-RT possessed some antioxidant activity.

The oxidizing substance can produce a free radical cation in ABTS, which is easily soluble in ethanol solution and has a significant UV absorption at 734 nm. When the antioxidant substance is added to it, the ABTS free radical cation can be partially eliminated and the absorbance of the solution can be reduced, so the antioxidant effect of the sample can be obtained according to the change of wavelength [28, 29].

As shown in Fig. 1D, the scavenging ability of SL-RT solid beverage increased with its concentration. 1.0 mg/mL of the solid beverage showed a scavenging rate of  $94.61 \pm 0.86\%$  of ABTS free radical, which was comparable to the scavenging effect of ABTS free radical at the same concentration of Vc, with good antioxidant activity. The activity trend is consistent with the concentration dependence of total phenol and total flavonoid contents, which conforms to the known action rules of polyphenolic components.

The DPPH scavenging rate (82.4%) and ABTS scavenging rate (94.6%) of the beverage increase with the rise in concentration, and this trend is consistent

with the concentration dependence of total flavonoid and proanthocyanidin contents. Existing studies have confirmed that flavonoids (such as quercetin) exert antioxidant effects through hydrogen atom transfer and metal chelation<sup>[30]</sup>, Proanthocyanidins enhance scavenging capacity through a synergistic effect. Therefore, it is speculated that these two types of polyphenols are the main material basis for the antioxidant activity of the beverage. The above results indicate that the antioxidant capacity of the beverage increases with the increase in the contents of total phenols and total flavonoids, confirming the core role of polyphenolic components.



**A: DPPH free radicals; B: total antioxidant activity; C: OH<sup>·</sup> free radicals; D: ABTS free radicals**

**Fig. 1 Antioxidant activity of solid beverages and Vc**

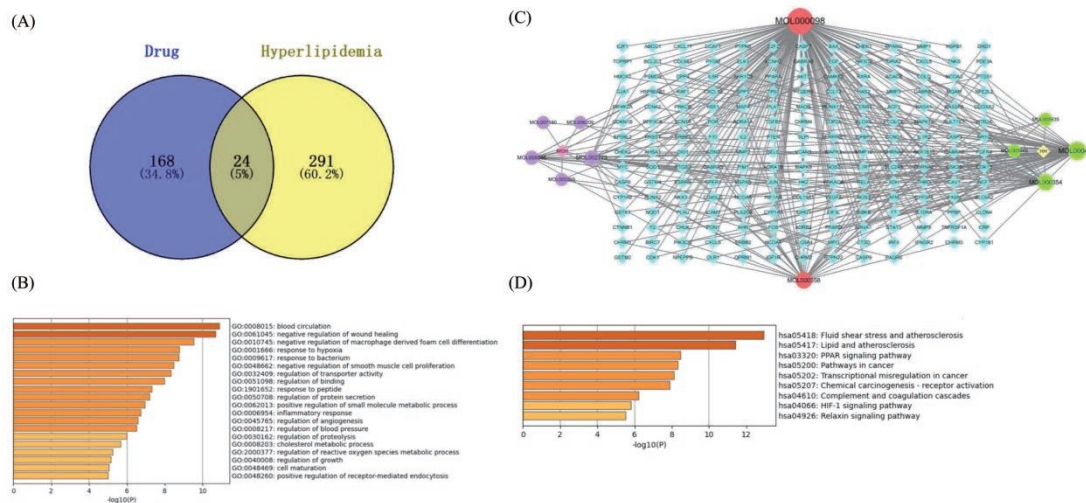
### 3.3 Lipid metabolism

Based on the TCMSP database, the active compounds of SL and RT were searched and the compounds with OB  $\geq 30\%$  and DL  $\geq 0.18$  were screened. The results showed that SL contained 5 compounds and RT contained 7 compounds, quercetin and  $\beta$ -sitosterol are the active compounds shared by

SL and RT. The results are shown in Supplementary B. A total of 192 target genes related to hyperlipidemia were obtained by searching the databases of OMIM, TTD, GeneCards and so on. The target genes and disease-related target genes of medicinal compounds were introduced into Venny 2.1.0 website, there were 24 overlapping targets (Fig. 2A). The blue nodes in the “medicine-component-target” interaction network

(Fig. 2B) number the active components of the drug (SL and RT), the red nodes represent the common active components (quercetin and  $\beta$ -sitosterol) of SL and RT. Each line represents the interaction between the active component and the target, and the degree is taken as the main reference for topological analysis. The results showed that quercetin, kaempferol,

$\beta$ -carotene and other compounds in SL and RT had the highest degree of association with the target, which may be the key compounds with therapeutic effects on hyperlipidemia. GO (Fig. 2C) and KEGG (Fig. 2D) bioconcentration analysis showed that they were responsible for fluid shear stress and arteriosclerosis, lipid pathways, etc..



**A: Venny diagram of medicine and hyperlipidemia; B: The network diagram of “medicine-component-target”; C: GO pathway enrichment analysis; D: KEGG pathway enrichment analysis**

**Fig. 2 Network Pharmacology Analysis**

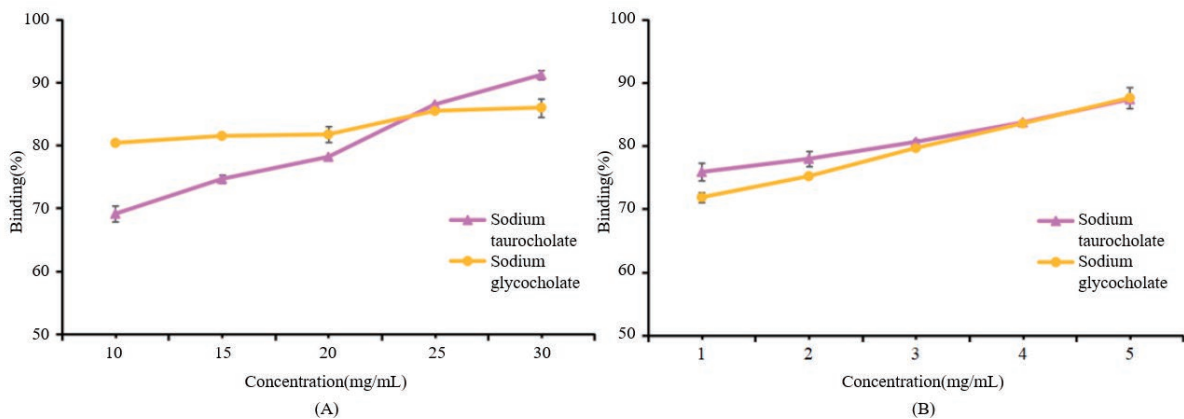
In the body, cholic acid combines with other inorganic salts, usually cholate. Cholesterol is involved in the synthesis of cholic acid, the reduction of cholic acid helps to reduce the formation of cholesterol, SL-RT solid beverage has the ability to bind sodium cholate, can reduce the reabsorption of cholic acid, promote the decomposition of cholesterol, to achieve the role of lipid-lowering<sup>[31,32]</sup>. Pancreatic lipase is a key enzyme for triglyceride uptake in lipid metabolism. Inhibition of lipase is thought to be an effective mechanism for inhibiting the uptake of triacylglycerides in patients with hypercholesterolemia<sup>[33-35]</sup>. From Fig. 3, it can be seen that the solid beverage and quercetin have adsorption effect on both sodium cholate. As shown in Fig. 4, the inhibition rate was gradually increased with increasing the concentration of SL-RT solid beverage samples, with an inhibition rate of 78.41% at 50 mg/mL and IC<sub>50</sub> (the half maximal inhibitory concentration)

value of 32.55 mg/mL, it has strong ability to inhibit pancreatic lipase. As can be seen in Fig. 4B, the lipase inhibition rate of quercetin at 0.6 mg/mL was 78.53% and IC<sub>50</sub> value of 0.2723 mg/mL. The monomer had pancreatic lipase adsorption activity and was significantly higher than that of SL-RT solid beverage. Network pharmacology shows that quercetin and  $\beta$ -sitosterol (polyphenol-related active components) can regulate lipid metabolism pathways (Fig. 2D). In *in vitro* experiments, the beverage’s bile salt adsorption capacity (589.4 ± 2.9 mg/g) and pancreatic lipase inhibitory effect (IC<sub>50</sub> = 32.55 mg/mL) are consistent with the known mechanism of flavonoids, i.e., “inhibiting lipase activity and blocking bile salt reabsorption”<sup>[36]</sup>, this further confirms the lipid-lowering contribution of polyphenolic components (especially flavonoids).

Cholesterol adsorption activity is also an important indicator for assessing the hypolipidemic

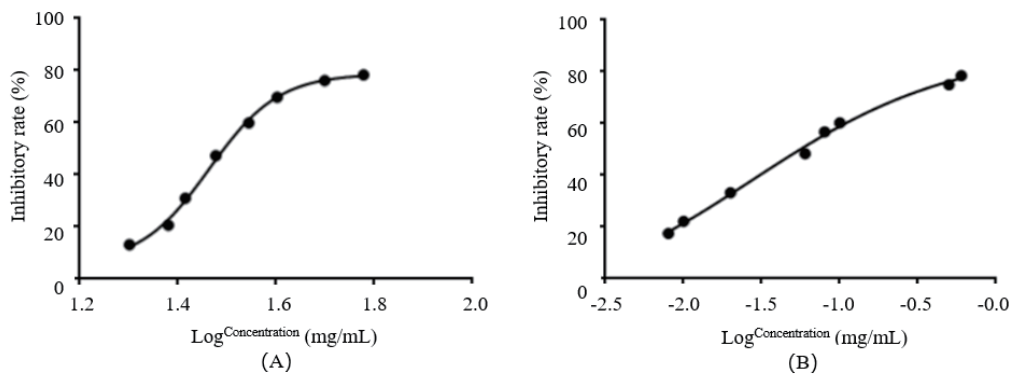
properties of SL-RT solid beverages<sup>[37]</sup>. SL-RT solid beverage can adsorb a certain amount of cholesterol, under acidic conditions, the cholesterol adsorption activity of solid beverages was  $227.9 \pm 2.4$  mg/g at pH 2.0, and the adsorption capacity was  $589.4 \pm 2.9$  mg/g at pH 7.0, acidic conditions on the adsorption of cholesterol is lower than the neutral conditions. It shows that SL-RT solid beverage can occur predominantly in the intestines rather than in the

stomach, effectively combine cholesterol, promote the role of lipid-lowering. Solid beverages through the combination of lipid, gastrointestinal digestion and absorption of lipid to reduce the amount of fat to achieve the goal of reducing fat<sup>[38]</sup>. The sorption activity of SL-RT solid beverage to soybean oil was  $0.77 \pm 0.15$  g/g. the results showed that the SL-RT solid beverage had certain sorption activity to soybean oil.



**A:** the binding rate of solid beverages to sodium taurocholate and sodium glycocholate; **B:** the binding rate of quercetin to sodium taurocholate and sodium glycocholate

**Fig. 3** The binding rate of cholates by different concentration of solid beverages and quercetin



**A:** SL-RT solid beverage; **B:** quercetin

**Fig. 4** The inhibitory rate of pancreatic lipase by different concentration of solid beverages and quercetin

### 3.4 Shelf life stability

The changes in the moisture content of the SL-RT solid beverage when stored at room temperature are shown in Fig. 5, and the overall changes showed a clear upward trend with the increase in time. At day 60, the

moisture content of the solid beverages at this time was  $4.87 \pm 0.15\%$ . Through the food standards, it is known that the moisture content of solid beverages does not exceed 5%. Therefore, the combined indicators yield that the solid beverages do not exceed 60 days under storage conditions at room temperature. The pH value

of the brewed SL-RT solid beverage showed a stable trend in general during the storage period of 60 days, with slight fluctuations around 5.15, which is weakly acidic and able to inhibit some microbial survival and growth, which is conducive to the storage of solid beverages. The dissolution time of the solid beverages did not change much within 14 days, and the particles were loose. After 14 days, the dissolution time became significantly longer, and the solid beverages became difficult to brew, and the dissolution time on the 60th day was already as high as  $30.88 \pm 1.01$  s. Long-term storage would reduce the quality of solid beverages, absorb water and clump together, resulting in difficult to be brewed. The sensory scores changed as shown in Fig. 6, with the increase of time, the sensory scores had a certain decreasing trend, the sensory acceptability was higher than the minimum threshold value of 60 during the storage period, until the sensory scores were as low as  $76.84 \pm 1.11$  at 60 days, the liquid was turbid after brewing, with a little suspension and precipitation, and the color was dark and dull, but it still had the distinctive fragrance of the SL-RT solid beverage. The color change graph is shown in Table 2.

The color difference value  $L^*$  of the solid beverages decreased significantly at the 30th day, and the brightness of the color became darker after brewing.  $a^*$  and  $b^*$  showed an overall increasing trend, indicating that the color of the solid beverages became darker towards brownish-yellow. These changes in the SL-RT solid beverage during storage are closely related to the properties of polyphenols (especially flavonoids) it contains. Polyphenols and flavonoids exhibit certain antioxidant properties. This characteristic allows the pH value of the beverage to remain weakly acidic, around 5.15, and relatively stable during the shelf life. Such stability plays a role in inhibiting the growth of microorganisms, which is beneficial for storage. However, due to their chemical reactivity, these compounds may participate in oxidation reactions and enzymatic browning during storage. This participation leads to changes in the beverage's color and a decline in its solubility, thereby affecting the shelf-life stability. This phenomenon indicates that although polyphenols and flavonoids confer certain advantages to the beverage, they also present challenges to its shelf-life stability.

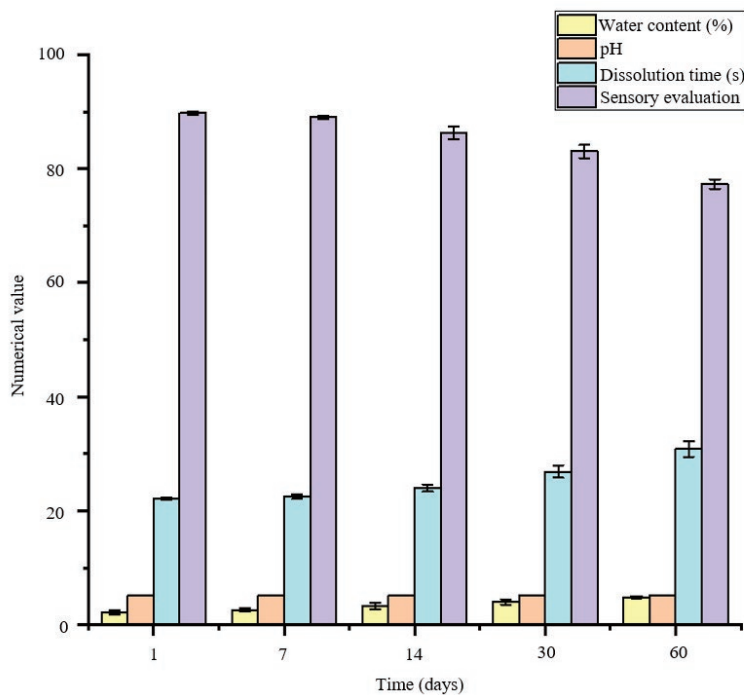
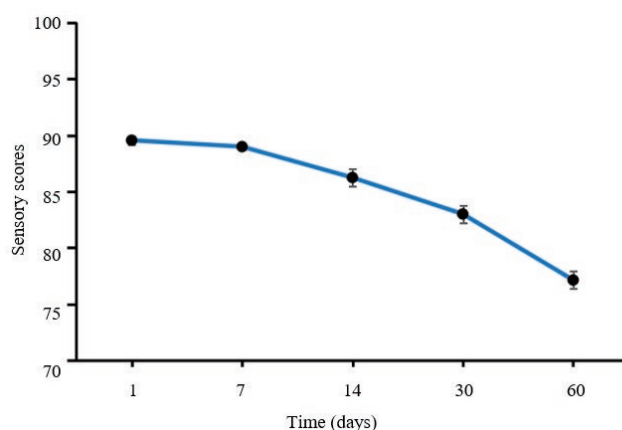


Fig. 5 Effect of storage time on water content, pH, dissolution time, and sensory evaluation of solid beverages



**Fig. 6 Effect of storage time on sensory evaluation of solid beverages**

**Table 2 Effect of storage time on color of solid beverages**

Time (days)	L*	a*	b*
1	57.37 ± 0.32	3.02 ± 0.22	16.90 ± 0.37
7	57.68 ± 0.38	2.46 ± 0.18	13.84 ± 0.40
14	59.01 ± 0.49	3.09 ± 0.25	17.83 ± 0.51
30	51.75 ± 0.25	3.43 ± 0.19	16.87 ± 0.21
60	50.15 ± 0.53	3.55 ± 0.33	17.31 ± 0.28

## 4 Conclusion

In summary, this study successfully developed an SL-RT composite solid beverage rich in polyphenols (with flavonoids as the core active component). The beverage showed good antioxidant and lipid-lowering properties, which were attributed to the bioactivities of polyphenols and flavonoids. However, the stability during storage was affected by the chemical reactivity of these compounds, leading to color changes and reduced solubility. Future studies could incorporate encapsulation technologies to protect polyphenols and flavonoids, or screen excipients with lower hygroscopicity to enhance stability and fully utilize the health-promoting potential of polyphenols and flavonoids. This study provides a reference for the development of stable, functional beverages.

In this study, qualitative and quantitative analyses of polyphenol monomers and subclasses (such as phenolic acids, flavonoid glycosides, and proanthocyanidin monomers) were not conducted.

However, the correlation between the measured contents of total phenols, total flavonoids, and proanthocyanidins and functional activities, as well as the confirmed role of flavonoids (e.g., quercetin) through known pathways via network pharmacology, have preliminarily verified the contribution of polyphenolic components to the beverage's functions. In subsequent studies, techniques such as HPLC-MS can be used to identify and quantify relevant polyphenol types, and combined with technologies like molecular docking, the "specific polyphenol-function" correspondence and the "component-target" mechanism of action can be clarified.

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