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Systematic preliminary test on the chemical components of *Malus halliana* Koehne Fruit

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Abstract
The present study carried out systematic preliminary test on the chemical components of the fruit of *Malus halliana* Koehne. Phytochemical studies on the extracts from water, 95% ethanol and petroleum ether showed the presence of flavonoids, alkaloids, organic acid, carbohydrates, sterides and triterpenes. It was preliminarily identified that there was various components in the fruit of *Malus halliana* Koehne and the fruit may be potential in exploration for the isolation and further treatment of diseases.

Keywords: Fruit of *Malus halliana* Koehne, Systematic preliminary test, Chemical components

1. Introduction
Malus halliana Koehne is an important raw material that contains wide varieties of active chemical components including flavonoids, organic acids, lipids, proteins, vitamins, mineral elements and so on [1]. Nowadays, the limited studies of *Malus halliana* Koehne were concentrated on cultivation management [2, 3] or processing technology for healthy drink [4-6]. As for chemical composition study, only volatile components from full flowers and buds were reported [7]. Experiments are focused on exploring the leaves and full flowers [8] and are mainly about anti-oxidant activity [9-11]. For inhibition activity of α-glucosidase, Feng [12] only explored the leaves of *Malus halliana* Koehne and isolated thirty-one compounds while Zhang [13] compared the leaves with the full flowers. The only article [14] about this fruit was about determining the flavonoids and phenolic components and its anti-oxidant activity, which is incomplete.
This research was conducted with an objective to discover new bioactive compounds by phytochemical screening of plant materials before investigating a new plant material. As part of continuing research of the fruit of *Malus halliana* Koehne, we investigated the phytochemistry in the fruit from different extracts in order to provide experimental foundation for the bioactive components and drug efficacy research of *Malus halliana* Koehne.

2. Materials and methods [15-20]
2.1 Collection of plant samples
The fruit was collected in September from Southwest University of Science and Technology, Mianyang, Sichuan, China and authenticated by the authority of Dr. Ma Lin from Engineering Research Center for Biomass Resource Utilization and Modification of Sichuan Province.

2.2 Extraction
The fresh fruit was washed before being dried in the constant oven at 50 ℃ for 48 h. Then, smashed and screened it through mesh size of 120 for further use.
Preparation of extracting solution was based on methods with some modification. Briefly, petroleum ether extract: 10.00 g powders with 100 mL of petroleum ether were extracted with ultrasonic in the conical flask at 60 ℃ for 30 min. Then the filtrates were concentrated for testing volatile oil and triterpenes. Ethanol extract: 10.00 g powders with 100 mL of 95% ethanol were extracted with reflux in the round-bottom flask at 80 ℃ for 45 min and were added with 95% ethanol to 100 mL in the end. The filtrates were divided into two samples. One was for detecting flavonoids compounds, sterides, triterpenes. The other was concentrated and dissolved with 1% HCl to 50 mL for detecting alkaloids. Water extract: 10.00 g powders with 100 mL distilled water were placed in the conical flask for 24-hour cold leaching. Then 10 mL was detecting amino acid, polypeptide and protein. The residues were heated at 70 ℃ for 30 min to detect carbohydrates and saponins.

2.3 Phytochemical Evaluation
By using following standard procedures, the samples were analyzed for the presence of major phytochemical constituents. According to the reaction phenomenon, the existence of phytochemical constituents in tested drugs could be judged.

3. Results and discussion
Several phytochemical screening tests on the fruit of *Malus halliana* Koehne in different solutions were carried out and analyzed. The following tables reported the results. From the result, flavonoids, sterides, triterpenes, carbohydrates, phenols, tannins and other components were significantly present in the fruit of *Malus halliana* Koehne, which indicated vital basis for the further research and clinical application. However, chemical components like anthraquinones, proteins and amino acid were not observed in the fruit. Quantitative analysis of the fruit and purity of crude extract need further study.

3.1 Preliminary test on petroleum ether extraction
The petroleum ether extracting solution was used for the detection of volatile oils, grease, sterides, triterpenes. Table 1 reported the results .

Table 1: Results of systematic preliminary tests of petroleum ether extraction

Detection item	Detection method	Index of positive reaction	Phenomenon	Detection Result
Volatile oils and grease	Oil spot examination	Oil spot volatilized at room temperature(volatile oils)	Oil spot volatilized at room temperature	+
	Phosphomolybdic acid test	Blue spot	Yellow background, no blue spot	-
	Vanillin-concentrated sulfuric acid experiment	Red spot, blue spot or purple spot	No	-
Sterides and triterpenes	Anhydride-concentrated sulphuricacid reaction	Yellow → red → purple → green	Yellow → red → light purple → blue green	+ +
	Salkowski Reaction	Red or cyan in chloroform layer, green fluorescence in sul-furic acid layer	Cyan in chloroform layer, green fluorescence in sul-furic acid layer	+ +

Note: "+" indicated positive reaction, "++" indicated significant positive reaction, and " - " indicated negative reaction.

3.2 Preliminary test on 95% ethanol extraction

The 95% ethanol extracting solution was used for the detection of flavonoids, alkaloids, organic acid, lactone, coumarins and so on. Table 2 reported the results .

Table 2: Results of systematic preliminary tests of 95% ethanol extraction

Detection item	Detection method	Index of positive reaction	Phenomenon	Detection Result
	HCl-Mg powder reaction	Pink or red	Yellow with foam, turning light red after heating	+ +
Flavonoids	Aluminium trichloride test	Yellow or yellow green fluorescence	Yellow fluorescence	+ +
	Lead acetate test	Turbidity or precipitation	Yellowish white precipitation	+ +
	Ammonia-fumigation test	Light yellow to yellow, fluorescence	Light yellow fluorescence	+ +
Alkaloids	Bismuth potassium iodide test	Light yellow or brownish yellow precipitation	Light yellow with some turbidity	+
	Silicotungstic acid test	Light yellow or white precipitation	Light yellow with some turbidity	+
	Bromophenol blue test	Yellow spot in blue background	Yellow spot in blue background	+
Organic Acid	Bromocresol green test	Yellow spot in blue background	Brighter yellow spot, no blue background	+
	pH test paper detection	pH < 7.0	pH value at about 4.6	+
Lactone, coumarins	Fluorescence experiment	Blue fluorescence spots, turn yellow-green after spraying potassium hydroxide	Blue fluorescence spots, turn yellow-green after spraying potassium hydroxide	+
	Alkali test	Turning red after adding alkali, red faded after acidification	Turning deep yellow adding alkali, yellow faded after acidification	-
Anthraquinones	Magnesium acetate reaction	Red	Light yellow with some turbidity	-
	1% Boric acid	Orange fluorescence	No	-
	Ferric chloride - potassium ferricyanide test	Blue spot	Blue spot	+
Phenols and tannins	Ferric chloride test	Green,blue or dark purple	Deep yellow	-
	NaCl-gelatin test	White precipitation or turbidity	No	-
Cardiac glycosides	Kedde Test	Purplish red	No	-

Note: "+" indicated positive reaction, "++" indicated significant positive reaction, and " - " indicated negative reaction.

3.3 Preliminary test on water extraction

The water extracting solution was used for the detection of phenols, tannins, organic acid, proteins and so on. Table 3 reported the results .

Table 3: Results of systematic preliminary tests of water extraction

Detection item	Detection method	Index of positive reaction	Phenomenon	DDetection Result
Phenols and tannins	Ferric chloride-potassium ferricyanide test	Blue spot	Blue spot	+ +
	Ferric chloride test	Green, blue or dark purple	Deep yellow green	+ +
	NaCl-gelatin test	White precipitation or turbidity	White turbidity	+
Organic Acid	Bromophenol blue test	Yellow spot in blue background	Yellow spot in blue background	+ +
	Bromocresol green test	Yellow spot in blue	Brighter yellow spot, no	+

		background	blue background	
	pH test paper detection	pH < 7.0	pH value at about 4	+ +
Saponins	Acetic anhydride-concentrated sulfuric acid test	Purple red	No	-
	Trichloroacetic acid test	Red spots by heating	No	-
	Phosphours molybdic acid test	Dark blue	Greyish-green spot	-
Proteins and amino acid	Biuret reaction	Purple, red or purple red	Deep yellow	-
	Precipitation reaction	Turbidity or precipitation	No	-
	Ninhydrin reaction	Blue or purple blue	No	-
Carbohyd-rates	Fehling's test	Red Precipitate	Red Precipitate	+ +
	Molisch's test	Violet color at the interface of two liquids	Violet color at the interface of two liquids	+ +

Note: "+" indicated positive reaction, "++" indicated significant positive reaction, and " - " indicated negative reaction.

4. Conclusion

Most of *Malus halliana* Koehne plants are used for cultivation, and the fruit resources are not fully utilized. Systematic preliminary test of the chemical components in the fruit of *Malus halliana* Koehne was carried out for the first time . It could be concluded that the fruit of *Malus halliana* Koehne had flavonoids, organic acids, alkaloids, lactone, coumarins, sterides and triterpenes phenols, tannins, carbohydrates and other components, which provided important scientific basis for the further research of *Malus halliana* Koehne. It showed that the fruit can be potential in disease treatment or being a food additive.

5. References

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