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Functional food design of dandelion salad with antibacterial properties

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Abstract

Dandelion is a wild herbaceous plant commonly consumed in the daily diet of Western countries. It contains large amounts of nutrients including minerals, iron, calcium, sodium, magnesium, and vitamins A, B, C, E, and K. It is also a traditional Chinese medicine with a wide range of pharmacological activities including antimicrobial. In traditional Chinese medicine, dandelion is "isogenic of medicine and food", which may be functional as food and medicine. "Chinese herbal salad" is a usual choice for the Chinese; but, it is difficult to classify its quality and quantity. The quality relies on the place of origin or method of cultivation, and the quantity depends on the amount of dandelion used in making Chinese herbal salad for a specific function, such as antibacterial properties. Therefore, the present work designed a functional food (Chinese herbal salad) using dandelion as the ingredient, and compared different cultivation methods (soil or hydroponic) in terms of their antibacterial properties. A questionnaire survey was also conducted on the dandelion salad to verify its acceptance and suitability for use in salad recipes. This project would develop a more diverse and innovative dandelion food available to the public, and promote the use of this herbal plant as a health and nutritional supplement in the daily diet.

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Introduction

Dandelion is the dried whole herb of *Taraxacum mongolicum* Hand.-Mazz., *T. borealisinense* Kitam., or any species in the *Taraxacum* genus. It is widely distributed, and grows on roadsides, fields, grasslands, hillsides, riverbanks, and sandy soil. It is one of the most commonly used traditional Chinese medicines for its ability to clear heat, promote detoxification, reduce swelling, dispel stagnation, induce diuresis, and treat stranguria. It has also been used to treat acute mastitis and swelling pain, furuncle toxin, scrofula, diarrhoea, sore throat, cold fever, jaundice, different kinds of infectious diseases, and a variety of inflammatory illnesses, like pharyngitis gastritis, cholecystitis, hepatitis, mumps, and conjunctivitis (CPC, 2015).

Nowadays, dandelion is a popular cooking ingredient, owing to its medical and nutritional values. According to Materia Medica for Famine Relief "*Jiu Huang Ben Cao*" (Zhu, 1406) and The Compendium of Materia Medica "*Ben Cao Gang Mu*" (Li, 1596), dandelion can be consumed as a kind of vegetable, and is typically served in the daily diet. It contains high nutrients including minerals like iron, calcium, sodium, magnesium, and vitamins A, B, C, E, and K (Schütz *et al.*, 2006; Zhou *et al.*, 2011; Escudero *et al*., 2013).

Chicoric acid and caffeic acid are the major active ingredients of dandelion, which are used as the marker in the quantitative analysis according to the Pharmacopoeia of the People's Republic of China 2020 (CPC, 2015). Dandelion also has antibacterial and anti-infectious activities due to flavonoids and polysaccharides and other phytochemicals including carotenoids, sesquiterpene lactones, sterols, and triterpenes (Mir *et al.*, 2013). Previous studies have demonstrated that dandelion exhibited antibacterial activity against various bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Proteus mirabilis* (Wang, 2014; Kenny *et al.*, 2015; Amin Mir *et al.*, 2016; Díaz *et al.*, 2018). The possible mechanism of dandelion on *Staphylococcus aureus* was suggested to induce bacterial death by depolarising and permeabilising cell membranes, as well as altering

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intracellular-enzyme activities of the bacteria (Xu *et al.*, 2021). Therefore, dandelion is proposed to be a potential natural antibacterial agent.

Growing evidence has demonstrated that dandelion possesses anti-oxidative, anti-inflammatory, antihyperglycemic, antiviral, anticancer, anticoagulant, and analgesic effects (Lis and Olas, 2019; Di Napoli and Zucchetti, 2021; Olas, 2022; Kania-Dobrowolska and Baraniak, 2022). Antioxidant eliminates free radicals, and suppresses oxidation by modifying the activities of the antioxidative enzyme, thus inhibiting lipid peroxidation, lowering reactive oxygen species production, and reducing cell death (Liu *et al.*, 2020; Yin *et al.*, 2022). The anti-inflammatory effect of dandelion is performed by inhibiting inflammatory response mediated by different pathways and cytokines, for example, NF-κB, TNF-α, IL-1β, and IL-6 (Zhang *et al.*, 2012; Xue *et al.*, 2017). Meanwhile, the antioxidation and anti-inflammation properties of dandelion are related to the interaction with various elements involved in glucose, lipid, and protein metabolisms which provide therapeutic effects to several metabolic dysregulations, such as diabetes (Wirngo *et al.*, 2016). It was reported that dandelion could also inhibit the growth of several kinds of viruses, like hepatitis B and influenza viruses (He *et al.*, 2011; Yang *et al.*, 2020). Dandelion could also suppress the proliferation and survival of colorectal and gastric cancer cells (Ovadje *et al.*, 2016; Zhu *et al.*, 2017).

Traditionally, plants are cultivated and grown in the soil. Hydroponic cultivation is increasingly being adopted and encouraged to avoid soil pollution due to anthropogenic activities, including the improper usage of chemical pesticides and fertilisers, and pollutants released to the environment by solid-fuel boilers, road transport, cement factories, metallurgical, and ferrous industries. Heavy metals consisting of copper (Cu), cadmium (Cd), chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) are accumulated in the soil, and absorbed by the plants leading to a food safety issue (Harja *et al.*, 2023). Bioaccumulation of heavy metals could cause serious health problems as they have great neurotoxicity, nephrotoxicity, hepatotoxicity, immunological toxicity, cardiovascular toxicity, skin toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity (Mitra *et al.*, 2022). Hydroponic cultivation is a benefit of agriculture, not only by avoiding antinutrient absorption of plants, but also enhancing the production yield and bioactive substance concentrations (Wimmerova *et al.*, 2022). There is another study that demonstrated the levels of β-carotene and lycopene to be higher in tomatoes grown hydroponically, indicating an up-regulated nutritional value from this system (Verdoliva *et al.*, 2021), which implies the quality and quantity of the plants are greatly enhanced in the hydroponic system than that in soil cultivation.

Presently, many wild vegetable resources are used to make salad, and dandelion is the most particular one due to its nutritional and medicinal values. Recently, herbal salad has become popular in the Western countries; but, it lacks food-specific function as a therapy. Therefore, the present work aimed to design a functional food (Chinese Herbal Salad) based on the soil or hydroponic cultivation of dandelion, and to compare its antibacterial effect between the two methods of cultivation. Typically, salad is defined as any raw vegetables combined with toppings, and served with a dressing. In the present work, a new type of dandelion salad was developed, and its acceptability and suitability among the population was surveyed.

Materials and methods

Experimental materials and reagents

Fresh hydroponic dandelion (Figure 1A) was obtained from HKSKH Bishop Hall Secondary School, and dried soil dandelion (Figure 1B) was purchased from Guangzhou Zisun Pharmaceutical Co., Ltd. *Escherichia coli* (*E. coli*) ATCC® 25922 was purchased from Thermo Scientific (USA). BD Difco™ nutrient agar was purchased from Fisher Scientific (USA). 6.0 mm diameter blank paper discs were purchased from Liofilchem Co. (USA). Formic acid and methanol were purchased from Sigma-Aldrich (USA). Deionised distilled water (DDW) was prepared by a Milli-Q Direct 8 water purification system (Merck Millipore, USA). All the other reagents used were of analytical grade.

Extraction method

Fresh hydroponic dandelion was dried (Figure 1C) overnight at 50°C, and the following extraction method was modified by the Pharmacopoeia of the People's Republic of China 2015 (CPC, 2015). Soil or hydroponic dandelion was first ground to powder (Figures 1D and 1E). Next, 1 g of soil or hydroponic dandelion powder was weighed and transferred to 15

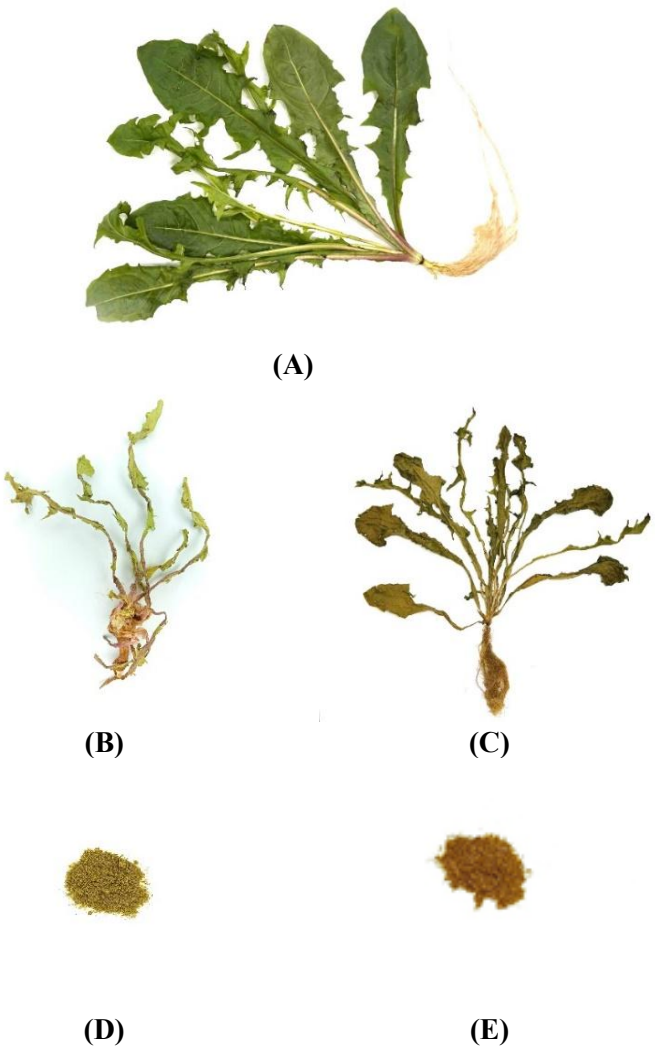


Figure 1. (A) Fresh hydroponic dandelion, **(B)** dried soil dandelion, **(C)** dried hydroponic dandelion, **(D)** dried soil dandelion powder, and **(E)** dried hydroponic dandelion powder.

mL graduated plastic Eppendorf centrifuge tube vial with a screw cap. Then, 10 mL of a methanol solution which contained 5% formic acid was added and shaken well. The centrifuge tube was put in an ultrasonic bath (250 W; frequency: 40 kHz) for 30 min, and an extractant was then filtered before use.

Antibacterial assay

Approximately 100 µL of the prepared *E. coli* suspension (~10^{-4}) was evenly spread on the Petri dishes containing 15 to 20 mL LB culture. Then, 100 µL of soil or hydroponic dandelion extract was dipped on the autoclaved paper disc, and placed onto the LB culture, which was incubated for 24 h at 37°C in a constant temperature biochemical incubator (Elbing and Brent, 2019). The production of an inhibition zone was observed, and the diameter of the inhibition zone (mm) was measured with a vernier calliper. This experiment was repeated three times.

Functional food design questionnaire survey

The functional food design questionnaire survey was conducted in this investigation. A total of 15 people, including students and staff from the Technological and Higher Education Institute of Hong Kong, Tsing Yi campus, participated. A Chinese Herbal Salad was designed focusing on dandelion, such as dried soil dandelion slices (Figure 2A) and hydroponic dandelion sauce (Figure 2B). The hydroponic dandelion salad (Figure 2C) was made up of vegetable and hydroponic dandelion sauce containing dandelion powder, sunflower seeds, and olive oil. The participants were required to taste the salad and dandelion salad before answering five questions in the provided questionnaire.

(A) **(B)** **(C)**

Figure 2. (A) Dried soil dandelion slices, **(B)** hydroponic dandelion sauce, and **(C)** hydroponic dandelion salad.

Statistical analysis

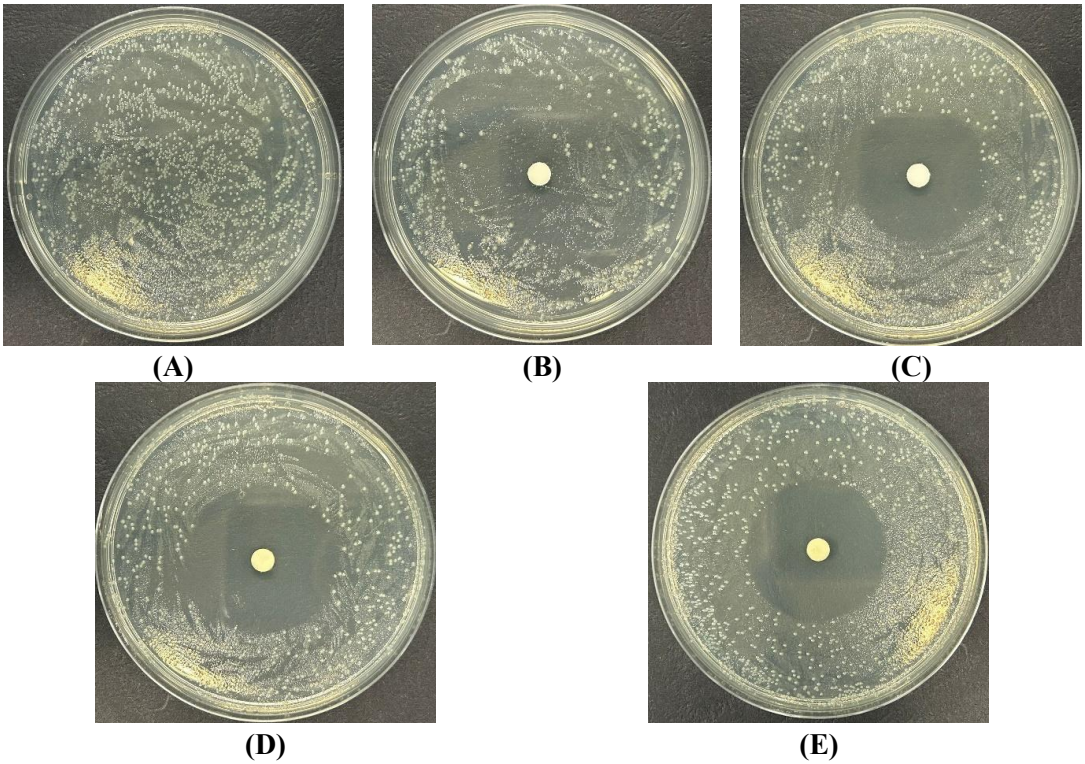
Statistical values were reported with the means of three separate experiments and the standard deviations (± SD). Results were subjected to Two-way ANOVA with SPSS software. Statistical differences at $p < 0.05$ were considered to be significant.

Results

Antibacterial assay

Five sets of experiments (Figure 3) on the disk diffusion method were performed to determine the antibacterial activity including three control groups: (A) *E. coli*; (B) *E. coli* and methanol; and (C) *E. coli*, methanol, and formic acid; while (D) soil cultivation of dandelion and (E) hydroponic cultivation of dandelion were the two sample groups.

Based on the results, the control groups of (B) *E. coli* and methanol showed no inhibition effect, but (C) *E. coli*, methanol, and formic acid did. This was a background comparison of the two sample groups, either in soil or hydroponic cultivation of dandelion. The antibacterial activity of hydroponic dandelion cultivation was nearly two-times greater (7.26%) compared to that of soil dandelion cultivation (3.74%) as shown in Table 1.



(A) **(B)** **(C)**

(D) **(E)**

Figure 3. Disk diffusion antibacterial assay activities: **(A)** *E. coli* (Control), **(B)** *E. coli* and methanol (Control), **(C)** *E. coli*, methanol, and formic acid (Control), **(D)** *E. coli* and dandelion (soil cultivation), and **(E)** *E. coli* and dandelion (hydroponic cultivation).

Table 1. Antibacterial activities of soil and hydroponic dandelion.

Group	*E. coli* and methanol (Control)	*E. coli*, methanol, and formic acid (Control)	*E. coli* and dandelion (soil cultivation)	*E. coli* and dandelion (hydroponic cultivation)
1	No inhibition effect	35.40 mm	35.60 mm	38.60 mm
2		34.70 mm	38.40 mm	37.70 mm
3		37.40 mm	37.50 mm	39.00 mm
Mean ($\bar{x}$) ± SD		35.83 ± 1.14 mm	37.17 ± 1.17 mm	38.43 ± 0.54 mm
Inhibition zone (%)*			3.74	7.26

*Inhibition zone (%) = [Mean (Sample – Control) / Mean of Control] × 100%.

Functional food design questionnaire survey

The functional food design questionnaire survey consisted of five questions, as summarised in Table 2. Generally, 87% of participants distinguished the dried dandelion slices salad, but 46.66% of them did not like it at all. Compared to the hydroponic dandelion sauce salad, most participants could not distinguish it, and above 66.67% of participants liked it. Overall, 73.34% of participants accepted the taste of a dandelion salad.

Mann-Whitney U and ANOVA tests were conducted in this questionnaire survey. Two combinations of questions were determined including Q1 *vs* Q2 and Q3 *vs* Q4 using the Mann-Whitney U test. Based on the findings, the *p*-value of these combinations were less than 0.05, which indicated that the results were significant with a relationship (Table 3). Other combinations of questions were determined including Q2 *vs* Q5, Q4 *vs* Q5, and Q2 and Q4 *vs* Q5 using the ANOVA test. Based on the findings, the *p*-value of these questions were greater than 0.05, which indicated that the results were insignificant (Table 4).

Discussion

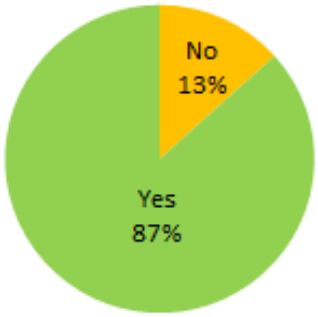
Dandelion is a natural antibiotic that possesses antibacterial activity. Zone of inhibition (ZOI) was a test conducted to determine the susceptibility or resistance of pathogenic bacteria to an antibacterial agent, for example, dandelion. The greater the inhibition zone, the higher the antibacterial activity that inhibited the growth of microorganisms. Hydroponic dandelion was better than soil dandelion, which showed nearly double the value of antibacterial activity. This might have been due to the active ingredients such as chicoric or caffeic acid present in higher contents in hydroponic dandelion, and these penetrated the bacterial cells, thus inducing cell death by damaging the membrane, protein, and DNA, and affecting the main functions of bacteria.

The functional food design for Chinese Herbal Salad was focused on the types of dandelion, either in hydroponic or soil cultivation relating to the function of dandelion on antibacterial activity, such as *E. coli*. It was expected to be able to inhibit *E. coli* within the human body when consumed in a daily diet, as well as to promote public health. The recipe and dosage of this hydroponic dandelion salad are important for its effectiveness against *E. coli*. Based on the findings, 46.66% of participants did not like the dried soil dandelion due to its bitter taste, and participants easily distinguish it. There were 66.67% of participants who liked the hydroponic dandelion sauce since the bitter taste was moderated by sunflower seeds. The hydroponic dandelion salad recipe contained 100 g vegetables, and three to four spoonful of hydroponic dandelion sauce containing 10 g dandelion powder, 10 g sunflower seeds, and 5 mL olive oil. The acceptance level for the taste of dandelion salad was higher than 73.34%. Thus, it was suitable to be promoted and commercialised to the public as a food therapy.

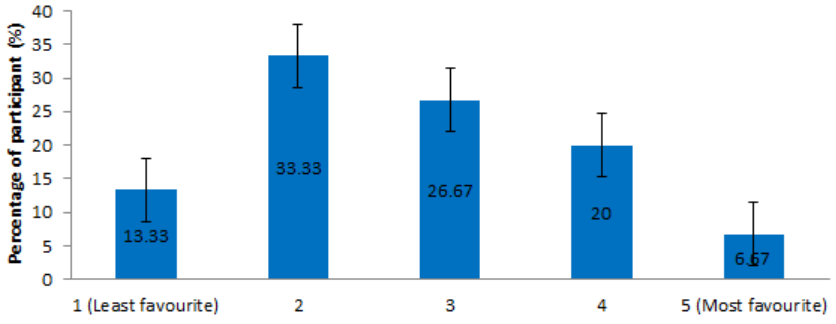
Besides, the Mann-Whitney U test was implied in this questionnaire survey for data analysis comparing differences between two independent groups, and determining its relationship between Q1 *vs* Q2 or Q3 *vs* Q4. Two combinations of questions in a total of 15 sample size with a given of 0.05 *p*-value were performed to estimate the significance of results. The *p*-value of these combinations were less than 0.05, and the results were significant with a relationship between them. Q1 and Q2, Q3 and Q4 were regarding the favourite of dandelion slices salad and dandelion sauce salad, respectively.

Table 2. Participants' responses to five questions in functional food design questionnaire survey.

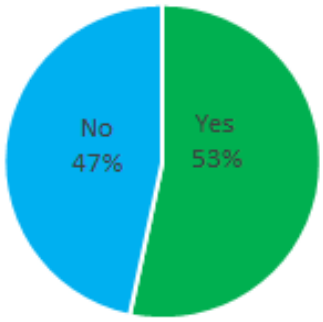
Q1. How many people can distinguish dandelion slices salad?



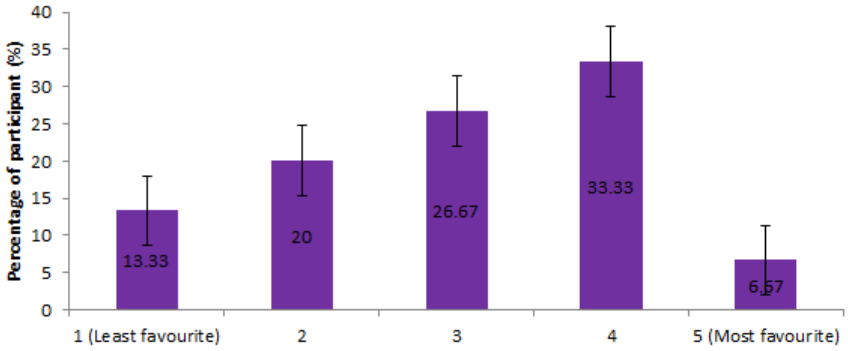
Q2. How much do you like dandelion slices salad?



Q3. How many people can distinguish dandelion sauce salad?



Q4. How much do you like dandelion sauce salad?



Q5. What is your acceptance level of dandelion salad?

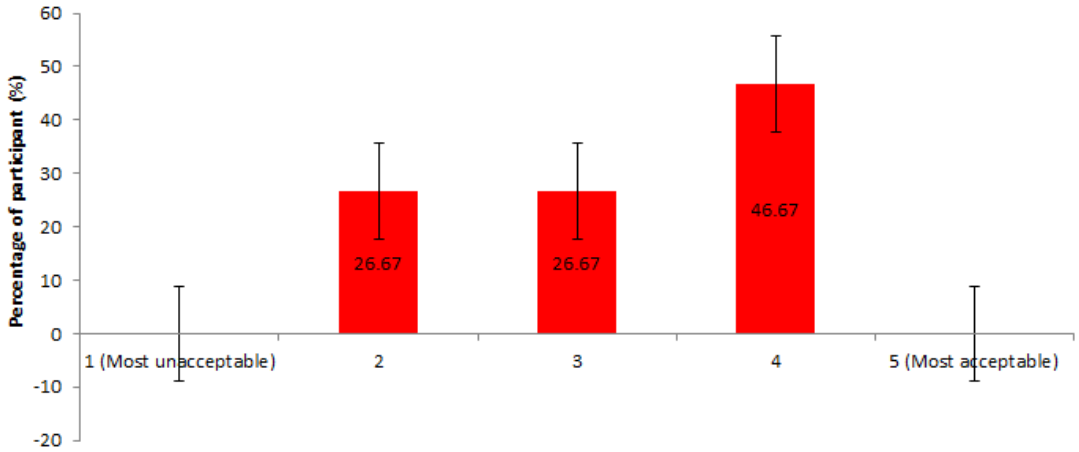


Table 3. Mann-Whitney U test for the comparison of significance for two combinations of questions.

Combinations of questions	**Q1 *vs* Q2**	**Q3 *vs* Q4**
z-value	-2.156860	-3.318245
p-value (0.05)	0.031017	0.000906
	$p < 0.05$ (significant)	$p < 0.05$ (significant)

Table 4. ANOVA test for the comparison of significance for two combinations of questions.

Combinations of questions	Q2 *vs* Q5	Q4 *vs* Q5	Q2 and Q4 *vs* Q5
F-value (3.10)	1.143	1.191	0.274
p-value (0.05)	0.475	0.461	0.842
	$p > 0.05$ (insignificant)	$p > 0.05$ (insignificant)	$p > 0.05$ (insignificant)

Another three combinations of Q2 *vs* Q5, Q4 *vs* Q5, and Q2 and Q4 *vs* Q5 were the determination of acceptable levels for dandelion, including dandelion slices salad and dandelion sauce salad. ANOVA test was used to compare the means of these three groups. The main research questions of these three combinations were: "Does the dandelion form affect the public's acceptance of it as a food ingredient?" It has three hypotheses based on these three combinations.

i. Hypothesis 1 (Q2 *vs* Q5): Acceptance of dandelion salad with dandelion slices. Null hypothesis (H_0): There is no significant difference in the acceptance of dandelion salad with dandelion slices compared to whole dandelion. Research hypothesis (H_1): Acceptance of dandelion salad depends on whether the dandelion was shredded.
ii. Hypothesis 2 (Q4 *vs* Q5): Acceptance of dandelion salad with dandelion sauce. Null hypothesis (H_0): There is no significant difference in the acceptance of dandelion salad with dandelion sauce compared to whole dandelion. Research hypothesis (H_1): Acceptance of dandelion salad depends on whether the dandelion was made into a sauce.
iii. Hypothesis 3 (Q2 and Q4 *vs* Q5): Dandelion salad with dandelion slices *vs* dandelion salad with dandelion sauce. Null hypothesis (H_0): The acceptance of dandelion salad is not related to the dandelion form either in slides or sauce. Research hypothesis (H_1): Acceptance of dandelion salad depends on the dandelion form either in slides or sauce.

Based on the results, the *p*-value of these hypotheses were greater than 0.05, which indicated that the results were insignificant. The dandelion form as a food ingredient did not significantly affect public acceptance. Whether it was sliced or made into a sauce, it did not impact the public's perception of dandelion as food, which identified that the market value of dandelion as a food ingredient remained constant, regardless of its form.

These results could help manufacturers develop various dandelion products in the future, such as salads with dandelion slices or dandelion sauce, without worrying about negative impacts on product acceptance. Additionally, consumers were more willing to try different dandelion food products since the dandelion forms were not a significant factor in determining their acceptance of the product. Dandelion has a certain value in the market, and is used as one of the daily foods in Chinese medicines. Hence, the conclusion may lead to the development of more diverse and innovative dandelion foods commercially available to the public, and promote the use of this herbal plant as a health and nutritional supplement in the daily diet.

Future aspect

The sample size of respondent was considerably small in this questionnaire survey. Thus, we only conducted the Mann-Whitney U and ANOVA tests. If the sample size was greater than 50, we could apply the *t*-test to obtain more precise and accurate results. Furthermore, the "quantitative" of dandelion salad must be well-defined, possibly by finding a marker for determining the effectiveness of its active ingredient in further investigation.

Conclusion

Dandelion is a wild herbaceous plant commonly consumed as Chinese herbal salad. Two types of dandelion cultivation, namely soil and hydroponic, were compared in the present work. The antibacterial activity of hydroponic dandelion was greater than that of soil dandelion. More importantly, the acceptance level of dandelion among consumers

was quite high, which can be a favourite salad ingredient used in salad recipes to cater to the market needs of wild resources.

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