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A Comparative Study of the Antioxidant Activities of Black Goji Berry *(Lycium Ruthenicum)* and Chinese Jujube *(Zizyphus Jujube Mill.)*, and their Combination

Ida Ayu Anom Rastiti[1*], Ida Ayu Suptika Strisanti[1], Putu Rima Sintyadewi[2]

[1]Bachelor of Acupuncture and Herbal Medicine, Institute of Technology and Health of Bali, Bali, Indonesia
[2]Bachelor of Food Technology, Institute Technology and Health of Bali, Bali, Indonesia
[*]e-mail: rastitiida@gmail.com

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Abstract: Oxidative stress, which arises from an imbalance between free radicals and the body's antioxidant defences, is a major factor in the development of degenerative diseases, making the search for natural antioxidants an important area of research. Black goji berry (*Lycium ruthenicum*) is known to be rich in anthocyanins and polyphenols, while Chinese jujube (*Ziziphus jujuba*) contains high levels of vitamin C, flavonoids, and polysaccharides; both have been traditionally used in herbal medicine and are considered promising natural antioxidants. This research is the first study that examines the synergistic antioxidant effect of black goji berry and Chinese jujube. An experimental laboratory study was conducted with five treatment groups consisting of Chinese jujube (100%); black goji berry (100%); black goji berry 75% : Chinese jujube 25%; black goji berry 25% : Chinese jujube 75%; and black goji berry 50% : Chinese jujube 50%. Infusion samples were analyzed for antioxidant activity, and data were further analyzed statistically using one-way ANOVA followed by Tukey's post hoc test. The results demonstrated significant differences in antioxidant activity among treatments ($p < 0.05$). Chinese jujube exhibited the weakest activity with an IC50 value of 35,695.24 ppm, while the 50:50 combination of black goji berry and Chinese jujube showed the strongest activity with an IC50 of 1,605.07 ppm. Groups containing black goji berry, either alone or in combination, consistently displayed better radical scavenging activity than Chinese jujube alone. These findings indicate that both black goji berry and Chinese jujube possess antioxidant potential; however, their combination, particularly in equal proportions, enhances this effect, suggesting a synergistic interaction. The results support the potential of these herbal mixtures as a complementary source of antioxidants to mitigate oxidative stress and prevent chronic diseases. They also highlight the value of studying plant combinations rather than single extracts.

Keywords: Antioxidant Activity; *Lycium ruthenicum*; Synergistic Effect; *Ziziphus jujuba*.

Introduction

Black goji berry, also known as black wolfberry and Chinese jujube, is a well-known medicinal plant widely used in traditional Chinese medicine. Both herbs are considered functional foods and herbal remedies, offering a range of health benefits. Black goji berry and Chinese jujube are highly recognized in traditional Chinese medicine due to their dual role as nutritious foods and medicinal herbs. Although each herb has been extensively studied, no research has specifically evaluated their combined antioxidant potential. This gap highlights the novelty of the present study, which investigates whether combining these two approaches can produce a synergistic effect.

Black goji berry (*Lycium ruthenicum*) is a medicinal plant distributed in China, Mongolia, and Central Asia. Its fruit has been traditionally used in Chinese medicine to treat various ailments such as heart disease, menstrual disorders, hypertension, urethral and urinary calculi [1], [2], [3]. These benefits are attributed to its functional components, such as anthocyanins, essential oils, organic acids, minerals, and polysaccharides. Anthocyanins are abundant in black goji berries and are the primary bioactive compounds functioning as antioxidants, cardiovascular protectants, and anti-tumor agents [4], [5], [6].

Chinese jujube (*Ziziphus jujube Mill.*) is a medicinal plant native to China and is also known as Chinese date [7]. This herb has been used for thousands of years in traditional Chinese medicine as well as a dietary supplement. In traditional Chinese medicine, it is used to alleviate fatigue, anorexia, diarrhea, and insomnia [8]. The fruit of the Chinese jujube is rich in vitamin C, flavonoids, and polysaccharides, which are natural antioxidants that help neutralize free radicals [9], [10], [11], [12] .

Black goji berry, which is rich in anthocyanins, and Chinese jujube, which contains high levels of vitamin C and flavonoids, represent a potential herbal combination worth further investigation. Their combination represents a promising candidate for enhanced antioxidant effects. The objective of this study is to analyze whether an infusion of black goji berry and Chinese jujube individually and in combination demonstrates synergistic antioxidant activity.

Research Methods

This study employed an experimental research design to evaluate the antioxidant activity (IC50) of black goji berry infusion, Chinese jujube infusion, and their combination. The research was conducted in several stages, beginning with the preparation of herbal materials, infusion

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preparation, sample treatment and antioxidant activity testing. This research was conducted at the Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University, over a two-month period from July to August 2025.

Sample Preparation

Black goji berries (*Lycium ruthenicum*) and Chinese jujubes (*Ziziphus jujube Mill.*) used in this study were sourced from certified suppliers with official distribution permits from the National Food Agency. Both dried fruits were prepared into infusions following Utami et al (2003) [13], by steeping 1 gram sample in distilled water at a ratio of 1:100 (w/v), followed by infusion at 60^0C for 60 minutes.

Experimental Design

Five variations of infusion compositions were prepared as treatment groups those are sample 1 (100% chinese jujube); sample 2 (100% black goji berry); sample 3 (75% black goji berry : 25% chinese jujube); sample 4 (25% black goji berry : 75% chinese jujube); sample 5 (50% black goji berry : 50% chinese jujube) as shown on table 1.

Table 1. The Combination of Black Goji Berry and Chinese Jujube Infusion

Material	Composition				
	S1	S2	S3	S4	S5
Black goji berry (ml)	0	100	75	25	50
Chinese jujube (ml)	100	0	25	75	50
Total (ml)	100	100	100	100	100

Note: S1(sample 1); S2 (sample 2); S3 (sample 3); S4 (sample 4); S5 (sample 5)

Antioxidant Capacity Measurement (DPPH Method)

The antioxidant activity of the samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Approximately 1–2 g of the sample was dissolved in methanol at various concentrations. From this solution, 1 mL was taken and mixed with 1 mL of DPPH solution at a concentration of 1,200 µM. The mixture was then incubated in the dark for 30 minutes to allow the reaction to occur.

After incubation, a reference solution consisting of 1 mL of DPPH and 4 mL of ethanol was prepared and used for calibration at a wavelength of 515 nm. The absorbance values obtained were used to calculate the percentage of antioxidant activity. A regression curve was then constructed by plotting sample concentration against the mean percentage of antioxidant activity, from which the IC50 value was calculated [13].

Data Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). For statistical analysis, the IC50 values obtained from different infusion formulations were compared using one-way Analysis of Variance (ANOVA). When significant differences were observed ($p < 0.05$), post hoc tests (Tukey's HSD) were applied to identify pairwise differences between treatment groups. All statistical analyses were conducted using IBM SPSS Statistics (version 27).

Results and Discussion

The antioxidant activity of black goji berry infusion, Chinese jujube infusion and their combinations was evaluated using the DPPH assay, and the IC50 values are summarised in Table 2. The result showed considerable variation among the samples. Sample 1 (100% Chinese jujube exhibits the highest IC50 value (35,692.24 ppm), indicating the lowest antioxidant activity. In contrast, sample 5 (50% black goji berry : 50% Chinese jujube) demonstrated the strongest antioxidant activity, with the lowest IC50 value (1,605.07 ppm).

Table 2. Antioxidant Activity of Black Goji Berry and Chinese Jujube Infusion and Their Combination

Composition	IC50 (ppm)
S1 (Chinese jujube 100%)	35,695.24 ± 1.01
S2 (black goji berry 100%)	7,557.04 ± 1.02
S3 (black goji berry 75% : chinese jujube 25%)	9,960.57 ± 1.09
S4 (black goji berry 25% : chinese jujube 75%)	23,334.13 ± 1.01
S5 (black goji berry 50% : chinese jujube 50%)	1,605.07 ± 1.01

The one-way ANOVA test revealed a statistically significant difference in antioxidant activity (IC50 values) among the five infusion compositions of black goji berry and Chinese jujube ($p < 0.05$). To further identify the source of these differences, a post-hoc analysis was conducted.

As presented in Table 3, all pairwise comparisons between the samples demonstrated significant differences ($p = 0.001$). The IC50 value of the 100% Chinese jujube infusion (S1) was significantly higher than those of the other groups, with the largest difference observed when compared with the 50:50 combination (S5; mean difference = 34,090.16 ppm). This finding indicates that Chinese jujube alone exhibits the weakest antioxidant activity among all other formulations.

Conversely, the 50:50 combination (S5) exhibited the lowest IC50 value and thus the strongest antioxidant capacity, showing significant differences from all other formulations. The mean differences between S5 and other samples ranged from 5,951.96 ppm (vs. S2, 100% black goji berry) to 34,090.16 ppm (vs. S1, 100% Chinese jujube).

Interestingly, when comparing the mixed formulations, the 75% black goji berry:25 % Chinese jujube (S3) combination also showed a significantly lower IC50 compared to the 25% black goji berry:75 % Chinese jujube (S4) combination, with a mean difference of -13,373.56 ppm ($p = 0.001$). These results suggest that increasing the proportion of black goji berries enhances the antioxidant activity of the infusion. The present study highlights the importance of formulation in maximizing antioxidant activity. The significantly lower IC50 values observed in the 50:50 mixture emphasize the potential of combining black goji berry and Chinese jujube as a functional beverage with enhanced health benefits.

Table 3. Mean Difference of Black Goji Berry and Chinese Jujube Infusion and Their Combination

Composition		Mean Difference (ppm)	P Value
S1	S2	28,138.19	0.001*
S1	S3	25,734.67	0.001*
	S4	12,361.10	0.001*
	S5	34,090.16	0.001*
S2	S3	-2,403.52	0.001*
	S4	-15,777.08	0.001*
	S5	5,951.96	0.001*
S3	S4	-13,373.56	0.001*
	S5	8,355.49	0.001*
S4	S5	21,729.05	0.001*

Note: *significant

The present study investigated the antioxidant activity of back goji berry (*Lycium ruthenicum*) and Chinese jujube (*Ziziphus jujube Mill.*) infusions, both individually and in various combinations. The one-way ANOVA followed by post-hoc analysis revealed statistically significant differences ($p < 0.05$) among all formulations, indicating that the antioxidant capacity was strongly influenced by the ratio of the two herbs. Among the tested samples, the 50:50 combination (S5) exhibited the lowest IC50 value (1,605.07 ppm), suggesting the highest antioxidant potential.

Black goji berry is recognized as an exceptionally rich source of anthocyanins, particularly petunidin-3-O-rutinoside and malvidin-3-O-glucoside, which exhibit strong free radical scavenging activity [14], [15]. In contrast, Chinese jujube contains abundant vitamin C, phenolic acids, flavonoids (e.g., quercetin, rutin), and polysaccharides, which contribute to antioxidant defence through multiple mechanisms, including direct scavenging of reactive oxygen species (ROS), metal chelation, and enhancement of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [16], [17]. These distinct phytochemical profiles suggest that the herbs may act through complementary antioxidant pathways.

Interestingly, the results showed that S3 (75% black goji berry : 25% Chinese jujube) had higher antioxidant activity compared to S4 (25% black goji berry : 75% Chinese jujube), reflecting the dominant role of black goji berry anthocyanins in lowering IC_{50} values. However, the best activity was observed in the 50:50 mixture (S5), which was superior not only to individual extracts (S1 and S2) but also to other combinations (S3 and S4). This observation supports the concept of phytochemical synergy, where the combination of anthocyanins from black goji berry and vitamin C or flavonoids from Chinese jujube interacts positively to enhance radical scavenging efficiency.

The possible mechanism is that vitamin C might regenerate oxidised anthocyanins and prolong their antioxidant action, while polysaccharides and flavonoids provide a stabilising matrix that reduces anthocyanin degradation. Anthocyanins neutralize free radicals primarily through hydrogen atom transfer (HAT) or single-electron transfer (SET) mechanisms. This action converts them into oxidized radical forms, which are less stable and lose effectiveness as antioxidants [18]. Vitamin C is a powerful antioxidant that mitigates oxidative stress by donating or transferring electrons. It can neutralize reactive oxygen, nitrogen, and sulfur species, while also playing a key role in restoring the activity of other antioxidants within the body [19]. Vitamin C (ascorbic acid) a potent reducing agent might donate electrons or hydrogen atoms to these oxidized anthocyanins, restoring them to their original, active form. This antioxidant recycling prolongs their radical scavenging capacity and enhances total antioxidant potential. Furthermore, a recent investigation confirmed that ascorbic acid helps protect anthocyanins from light-induced degradation, acting against peroxide radicals and thereby preserving anthocyanin integrity [20]. Such interactions may explain the enhanced activity of the 50:50 mixture.

Interactions among phytochemicals in multi-component systems may be synergistic, additive, or antagonistic, and the direction and magnitude of the effect often depend on the relative proportions of each component in the mixture [21] [22], [23] . Phytochemical interactions in multi-component systems can lead to synergistic effects depending on the proportion of each component. In the present study, the equal proportion of black goji berry and jujube likely provided the optimal balance for synergism.

The synergistic effect observed suggests that multi-herbal approaches could be superior to single-herb interventions. From a pharmacological perspective, such synergy may also enable the use of lower doses of each herb, thereby reducing the risk of potential side effects or adverse interactions. Beverages or supplements formulated with both black goji berry and Chinese jujube may offer enhanced functional benefits, appealing to consumers seeking natural antioxidants for maintaining their health.

From a functional food perspective, these results are important. The significantly lower IC50 value of the 50:50 mixture indicates that blending black goji berry and Chinese jujube could be a promising strategy for developing antioxidant-rich beverages or nutraceutical formulations. Moreover, the combination may offer broader health benefits, since anthocyanins are linked to neuroprotection and cardiovascular health [24], while jujube polysaccharides and vitamin C are associated with immunomodulatory and anti-inflammatory effects [25].

Overall, the present study highlights the importance of formulation in maximizing antioxidant activity. Although the 50:50 combination (S5) exhibited the lowest IC50 value among all formulations, indicating relatively higher antioxidant activity, the absolute value (1,605.07 ppm) is still considered weak when compared to standard antioxidants such as ascorbic acid, which typically display IC50 values in the range of a few µg/mL. Several factors may account for this low activity. First, the infusion method using distilled water may have limited the extraction efficiency of key phytochemicals, as anthocyanins and certain phenolic compounds are more soluble in organic solvents. Second, the concentrations of bioactive compounds in the dried fruit samples may not have been sufficiently high to exert strong radical scavenging activity at the tested dilution ratios.

Taken together, these results suggest that while the combination of black goji berry and Chinese jujube demonstrates some degree of synergism, the overall potency remains modest. Thus, these infusions may be more appropriately positioned as complementary sources of natural antioxidants rather than primary agents. Future studies should consider optimizing extraction methods (e.g., varying solvent polarity, temperature, or extraction time) and

exploring formulation strategies that preserve phytochemical integrity to enhance antioxidant activity to levels that are more comparable with standard antioxidants.

Conclusion

This study provides the first evidence that equal proportions (50:50) of black goji berry (Lycium ruthenicum) and Chinese jujube (Ziziphus jujube Mill.) produce a synergistic effect in enhancing antioxidant activity, although the overall potency (IC50 = 1,605.07 ppm) remains modest compared with standard antioxidants such as ascorbic acid. The complementary interactions between anthocyanins, vitamin C, flavonoids, and polysaccharides make this equal-ratio mixture superior to individual extracts or other combinations, underscoring the importance of formulation in determining functional outcomes. From a practical perspective, these findings highlight the potential for developing herbal drinks or nutraceutical products with enhanced functional properties that appeal to health-conscious consumers. Future research should aim to validate these synergistic effects in vivo, investigate a broader range of formulation ratios, and optimize extraction methods to preserve phytochemical integrity and maximize antioxidant potency, thereby advancing the development of functional foods and nutraceuticals based on black goji berry and Chinese jujube.

Author's Contribution

All authors contributed significantly to this study. Ida Ayu Anom Rastiti: conceptualization and study design. Ida Ayu Suptika Strisanti: Methodology development and experimental work. Putu Rima Sintyadewi: Data collection and analysis, with statistical analysis support from Author 3. All authors have read and approved the final version of the manuscript.

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