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Synergism test on dates (*phoenix dactylifera* l.) Fruit extract and soybean (*glycine max* (l.) Merr.)) Nuts extract on antioxidant activity

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Abstract

The exposure of sunlight containing UV radiation may trigger the formation of free radicals, namely ROS (Reactive Oxygen Species) which lead to various skin problems. Antioxidants play important roles in protecting the skin by stabilizing free radicals which cause skin damage. Dates (*Phoenix dactylifera* L.) and soybeans (*Glycine max* (L.) Merr.)) were reported to contain several natural antioxidants such as polyphenols, flavonoids, and isoflavones. The samples used in this study were ethanolic extracts of dates and soybeans. The Ferric Reducing Antioxidant Power (FRAP) method using a UV-Vis spectrophotometer was applied to evaluate the antioxidant activity. The results of the phytochemical screening showed that both samples contained phenolic compounds marked by a change in the color of the extract to a black color and flavonoid compounds indicated by the presence of an orange color. The results of antioxidant activity expressed in % FRAP activity were 160.508% for date fruit extract and 123.851% for soybean extract. The combination of these two extracts was indicated to be synergistic according to the calculation of the difference percentage, which was 60.273%. Date fruit extract has higher antioxidant activity than soybean extract and the combination of the two has a synergistic effect.

Keywords: Dates (*Phoenix dactylifera* L.); Soybean (*Glycine max* (L.) Merr.); FRAP; UV rays; Synergism

Introduction

Ultraviolet (UV) light is a type of light that comes from the sun. Radiation from UV light cannot be seen but can be felt by humans (Liana *et al*., 2021). Too frequent exposure to sun-containing UV rays will cause skin damage, such as erythema, pigmentation, photosensitivity, and premature aging (Rahmawati *et al*., 2018). This skin damage is caused by ROS namely, environmental exposure that triggers the formation of free radicals (Andarina & Djauhari, 2017).

Antioxidants are an alternative to inhibit the oxidation process and protect cells from damage caused by free radicals, even if only in small concentrations (Martemucci *et al*., 2022). Several natural components that act as antioxidants to protect the skin include polyphenols, monoterpenes, flavonoids, carotenoids, organosulfides, indole, chromanol, and chromenol. This compound has been proven to stimulate immune responses, be anti-inflammatory, modulate antioxidants, detoxify cells and tissues, change gene expression, and protect skin from aging (Pourzand *et al*., 2022).

Combining the two extracts containing antioxidants can produce synergistic, additive, or antagonistic interactions (Marianne *et al*., 2018). *Synergistic* is defined as the combined effect of two or more compounds that is greater than the sum of the individual effects. An additive effect is generally considered the combined effect of two or more compounds equal to the sum of the individual effects. In contrast, an antagonistic effect is observed when the effect of two or more substances in combination is smaller than the sum of the individual effects of the substance (Basavegowda & Baek, 2021).

The aim of carrying out this research is so that researchers can determine the antioxidant activity of date extracts, soybeans, and their combination, as well as the synergistic effect produced by the FRAP method, and provide recommendations regarding the selection of natural antioxidant sources that can be used as an alternative to the use of synthetic antioxidants, as well as ingredients. Raw material for entrepreneurial innovation that is useful as an antioxidant.

Methods

Plant determination test

The dates used were subjected to a determination test at the National Research and Innovation Agency (BRIN), Bogor. Meanwhile, the soybeans used were subjected to a determination test at the Department of Biology, University of Indonesia, Faculty of Mathematics and Natural Sciences, Depok, West Java.

Preparation of Sample Extract

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Sukkari dates are washed and separated from the seeds, then dried in an oven at 60°C for 24 hours. Dried simplicia powder of dates was weighed as much as 500 mg and macerated for 2 x 24 hours using 70% ethanol with a ratio of 1:2. The maceration results were filtered and concentrated using a rotary evaporator at 40°C with 80 rpm. Soybeans are washed and separated from the epidermis, then dried in an oven at 60°C. 250 mg of dried fruit simplicia powder was weighed and macerated for 2 x 24 hours using 70% ethanol with a ratio of 1:10. The maceration results were filtered and concentrated using a rotary evaporator at 40°C with 80 rpm.

Phytochemical Screening

The flavonoid test of extracts of dates and soybeans was carried out by mixing the extract with 5 mL of concentrated HCl and adding Mg powder. The presence of flavonoids can be seen in the formation of red, yellow, or orange colors (Nur, 2022). The phenol test for extracts of dates and soybeans was carried out by mixing the extract with 3-4 drops of 1% FeCl. The presence of phenolic compounds can be seen from the formation of a bluish-black color (Azizah & Wati, 2018).

Preparation of Acetate Buffer

Acetate buffer with a pH of 3.6 is made from 0.155 grams of sodium acetate trihydrate ($CH_3COONa.3H_2O$) added with 0.8 mL of concentrated acetic acid and dissolved with Aquadest to exactly 50 mL in a measuring flask.

Preparation of 2,4,6-tripiridyl-striazine (TPTZ) Solution

TPTZ was weighed at 30 mg and dissolved in 40 mmol/L HCl to exactly 10 mL. A 40 mmol/L HCl solution dissolves 0.1656 mL of concentrated HCl in 50 mL Aquadest.

Preparation of $FeCl_3.6H_2O$ Solution

A total of 0.27 mg $FeCl_3.6H_2O$ was dissolved with acetate buffer in a volumetric flask to exactly 50 mL.

Preparation of FRAP Reagent

The FRAP reagent is prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of FeCl3.6H2O solution. Then, add distilled water to precisely 100 mL in the measuring flask.

Preparation and Testing of $FeSO_4.7H_2O$ Standard Solution

50 mg of FeSO4.7H2O was dissolved in 50 mL of distilled water in a volumetric flask until a concentration of 1000 ppm was obtained. A concentration series of 50, 70, 100, 130, and 140 ppm was prepared in a 10 mL volumetric flask. From each concentration, 100 µl was taken, and 3 mL of FRAP reagent was added in a 5 mL measuring flask, homogenized, and stored in a brown vial. The solution was incubated at 37°C for 10 minutes. The absorbance of each concentration was read using a spectrophotometer with the maximum wavelength of the optimization results.

Maximum Wavelength Determination

100 µl of FeSO4.7H2O solution with a concentration of 140 ppm was added with 3 mL of FRAP reagent in a 5 mL measuring flask, homogenized. Furthermore, stored in a brown vial. The solution was incubated at 37°C for 10 minutes. Scan the solution at a wavelength of 580-610 nm.

Determination of Antioxidant Activity

100 µl of sample solution was added to 3 mL of FRAP reagent in a measuring flask 5 mL, homogenized, and stored in a brown vial. The solution was incubated at 37°C for 10 minutes. Then, 1 mL of the solution was taken and diluted to a volume of 10 mL in a measuring flask. Each sample was read for absorbance at the maximum wavelength. Antioxidant activity was obtained from absorbance data against the standard series FeSO4.7H2O and was expressed as equivalent to ppm Fe2+. The percentage of FRAP activity is calculated based on a formula.

FRAP activity (%) = (A/B) x 100 … (1)

A: control absorbance, B: sample absorbance

Antioxidant Activity Synergism Test

A total of 10 mg of date fruit sample was dissolved in distilled water to 10 mL (1000 µg/mL). A total of 20 mg of soybean sample was dissolved in distilled water to 10 mL (2000 µg/mL). From this concentration, 5 mL of each was taken and homogenized in a 10 mL volumetric flask. Take 0.1 mL and add 3 mL of FRAP reagent. Next, the absorbance was read at the maximum wavelength using a UV-Vis spectrophotometer. The synergism test is determined by calculating the % difference using the formula.

Difference (%)= [(Combination ab x 100)/((Individual a + Individual b)÷n)]-100 … (2)

Differences : Effects resulting from a mixture of extracts a and b,
Combination ab : Antioxidant activity (Fe2+ levels) in a mixture of extracts a and b,

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Individual a/b : Antioxidant activity (Fe2+ levels) + in extracts a/b,
N : Number of compounds in the mix.

Results and Discussions

The purpose of the determination test is to prevent sample errors and guarantee the correct identification of plants based on their taxonomy (Nurulita *et al.*, 2019). Test results show that this research used sukkari date fruit with the Latin name *Phoenix dactylifera* L of the Arecaceae family. Meanwhile, soybeans have the Latin name *Glycine max* (L.) Merr., Fabaceae family. The result of date fruit extract is a thick extract, brownish-yellow in color, and has a distinctive smell of dates. The result of soybean extract is a thick extract, brownish-yellow in color and odorless. The result yields were 20.92% and 8.4% respectively. The results of the acquisition of water content from date fruit extract and soybean extract were 7.17% and 0.46%, respectively.

Table 1. Results of Phytochemical Screening

	Flavonoid Test		Phenol Test	
Extract	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
Dates	Yellow clear	+ (Orange)	Orange	+ (Blackish brown)
Soybean	Yellow	+ (Orange)	Brownish yellow	+ (Blackish Green)

The phytochemical screening carried out on date fruit extract and soybean extract were the flavonoid test and the phenol test. In the flavonoid test, both samples produced an orange color. While the phenol test produced blackish brown and blackish-green. This shows that the positive sample contains flavonoid and phenol compounds.

Determining the maximum wavelength is done by looking at the absorbance standard curve of $FeSO_4.7H_2O$ at the highest concentration of 140 µg/mL in the range of 580-610 nm. The absorbance obtained is 0.782 at a maximum wavelength of 595 nm. The maximum wavelength spectrum is shown in **figure 1.**

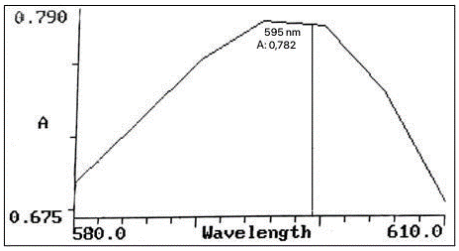

Figure 1. Maximum wavelength spectrum

Determination of standard curve absorbance in series solutions with concentrations of 50 µg/mL, 70 µg/mL, 100 µg/mL, 130 µg/mL, and 140 µg/mL resulted in the regression equation Y=0.0032x + 0.1638 with a value r2=0.999. The absorbance data obtained was used to calculate the % difference in the synergism test. The standard curve graph for $FeSO_4.7H_2O$ is shown in **figure 2.**

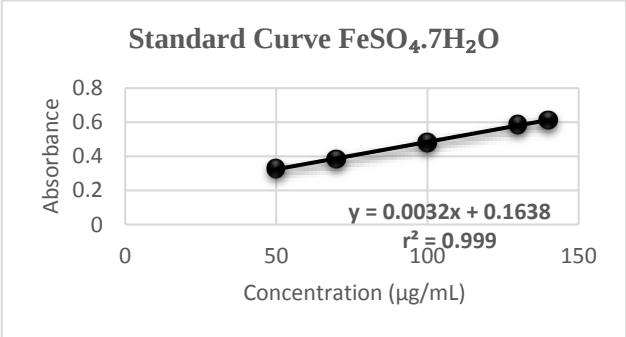

Figure 2. Standard curve graph

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Antioxidant activity in single extracts of dates and single extracts of soybeans (F1 and F9) as well as their combination (F2-F9) is expressed in % FRAP activity and Fe^{2+} content. The antioxidant activity of a single extract of dates obtained a Fe^{2+} level of 67.771 with a FRAP activity of 160.508%. While the activity of soybean extract obtained Fe^{2+} levels of 102.979 with FRAP activity of 123.851%.

Table 2. FRAP Activity % Value

Formulas	Treatment		Fe^{2+} level (µg/mL)	FRAP Activity (%)
	Date Fruit Extract (%)	Soybean Extract (%)		
F1	100	0	67,771	160,508
F2	80	20	56,938	176,590
F3	60	40	54,958	179,882
F4	55	45	82,979	142,314
F5	50	50	71,208	156,000
F6	45	55	73,188	153,518
F7	40	60	75,479	150,740
F8	20	80	60,271	171,308
F9	0	100	102,979	123,851
F10	100	100	136,833	101,551

The synergism test was carried out by mixing date extract and soybean extract in a ratio of 100:100. In calculating the % difference to determine the synergistic effect of the antioxidant activity of the samples, data is needed regarding the levels of antioxidant activity in a single sample extract. The sample synergism test value obtained a difference of 60.273%.

Table 3. synergism test

Formulas	Treatment		Fe^{2+} level (µg/mL)	Difference (%)
	Date Fruit Extract (%)	Soybean Extract (%)		
F1	100	0	67,771	-
F9	0	100	102,979	-
F10	100	100	136,833	60,273

In general, the weight of dates is 2-60 grams and 3-7 cm long (Azkiyah & Rahimah, 2022). The type of date studied was the Sukkari date, which has a light brown color and ripe yellow at the ends (Fandi, 2020). In general, soybean seeds have an oval, round, or flat shape, measuring 6-30 g per 100 seeds. Soybean seeds, which are wrapped in a seed coat, have two pieces called lesta (Stefia, 2017). Soybeans of the Anjasmoro variety were chosen as the research sample. Anjasmoro soybeans have large seeds (14.8-15.3 g/100 seeds), have the potential to obtain high yields of 2.3 tons/ha, and are often used as an essential ingredient in making tempe and tofu (Tambunan *et al*., 2020).

The assessment of a sample using human senses as the main tool is called organoleptic testing (Gusnadi et al., 2021). The focus of testing is color, shape, taste, and smell (Handoyo & Pranoto, 2020). Sukkari date pulp extract is a thick extract, brownish yellow, and has a distinctive date smell. This follows research conducted by Rani (2021), with the same sample forming a black-brown viscous extract with a distinctive smell of dates. Another study conducted by Azkiyah & Rahimah (2022) produced a thick extract, dark brown, and had a sweet smell typical of dates and a sweet taste. The color difference in the extract depends on the color of the date fruit powder obtained.

The results of the organoleptic test carried out on soybean extract were in the form of a thick extract, brownish yellow, and odorless. The yield value obtained indicates the number of components of the bioactive compounds contained in the sample extract (Senduk *et al*., 2020). From the thick extract of dates, the yield was 20.92%. Research conducted by Rani (2021) obtained a percent yield of date fruit extract of 8.82%. The results obtained are lower because of the difference in the solvent used, namely methanol, in the extraction process. Apart from that, there is a difference in the water content of the date extract, which is less, namely 5.88%, caused by the drying process using a high temperature, namely 80℃ for 10 hours. The total soybean thick extract obtained a percent yield value of 8.4%. The yield results obtained are in line with the research of Hasanah *et al*. (2019), namely 8.1% in soybean extract with the anjasmoro variety. The water content test obtained has met the quality requirements from The Indonesian Food and Drug Authority (BPOM), namely <10% (Wijaya, 2022). The high water content in simplicia causes damage to the compounds contained in the simplicia because it becomes a medium for the growth of fungi and bacteria (Santoso *et al*., 2022).

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Phytochemical screening is carried out using specific reagents and observing the color changes that occur (Vifta & Advistasari, 2018). Phytochemical screening of date and soybean fruit extracts was conducted, namely testing for flavonoid and phenolic compounds. Research with samples of date fruit extract and soybean extract resulted in a change in color to orange and positively contained flavonoid compounds. Research by Ramayani *et al.* (2021) showed a color change to orange after the sample was reacted with concentrated HCl and Mg powder so that it positively contained flavonoid compounds. The results obtained from the phenol test of date and soybean extracts were blackish brown and blackish green. This is in line with Nazilah's (2019), with ajwa date fruit extract producing a black-green color, and Rani's research (2021) using sukkari date fruit extract producing a black color after being reacted with $FeCl_3$.

Flavonoid and phenolic compounds are the largest group of phenolics in plants that are beneficial to health as antioxidants (Siddeeg *et al*., 2019). The hydroxy groups on the aromatic rings of flavonoids and phenolic compounds are easily oxidized so that they can provide their hydrogen atoms to free radicals (Dhurhania & Novianto, 2019). The ability of phenolic compounds to donate their hydrogen atoms can neutralize free radicals so that the chain radical reactions that are occurring can be stopped. Meanwhile, hydrogen atom donors in flavonoids play a role in inhibiting the formation of free radicals and preventing oxidation processes by chelating metal ions (Liza *et al*., 2020).

The determination of the maximum wavelength is carried out to determine the absorption area that can be generated in the form of absorbance values from the standard solution using a UV-Vis spectrophotometer (Sukmawati, 2018). The most excellent sensitivity and the best absorption of the test solution will occur at the maximum wavelength, resulting in optimal sample absorbance measurements (Wahid *et al*., 2017). This study used the 580-610 nm range for standard $FeSO_4.7H_2O$ at a concentration of 140 µg/mL to determine the maximum wavelength (Nurulita *et al.*, 2019). The highest absorbance value was 0.782, obtained at a wavelength of 595 nm. This follows research by Nurhayati *et al.* (2022) in determining the maximum λ using the standard $FeSO_4.7H_2O$ obtained a maximum wavelength of 595 nm.

The sample concentration can be determined by looking at the relationship between the absorbance value of the standard curve solution and its concentration in determining the standard curve (Suharyanto & Prima, 2020). The standard in this study used $FeSO_4.7H_2O$. This is related to the FRAP method reaction, which has a working principle, namely that antioxidant compounds are able to reduce Fe3+ ions to Fe2+ ions. $FeSO_4.7H_2O$ has Fe2+ ions, which can be used as an antioxidant standard. The determination of the standard curve was made in series solutions with concentrations of 50 µg/mL, 70 µg/mL, 100 µg/mL, 130 µg/mL, and 140 µg/mL. This is in accordance with research by Rumpf *et al.* (2023), which explains the standard curve range used, namely 40-190 µg/mL. A series of standard curves were created, and then the absorbance was read using a UV-Vis spectrophotometer. The absorbance data obtained is processed into a linear regression equation. The linear regression equation obtained is Y = 0.0032x + 0.1638, with a value of r2 = 0.999. The r2 value or correlation coefficient close to 1 shows that this regression equation is linear, so absorbance and concentration have a very strong correlation (Asmorowati & Lindawati, 2019).

Measurement of antioxidant activity in samples used a concentration of 1000 µg/mL for ethanol extract of dates and 2000 µg/mL for soybean extract. The sample absorbance value must enter the standard curve absorbance range, 0.328-0.611. Both extracts were formulated (F1-F10). Based on the FRAP activity values presented in Table 2, it is known that the single extracts tested, namely F1 and F9, obtained different FRAP activity values. F1, which is a date fruit extract, has higher FRAP activity than soybean extract, with an FRAP activity value of 160.508, while F9, which is a single extract from soybeans, has an FRAP activity value of 123.851. This follows research conducted by Nafisah (2019) by determining antioxidant activity using the DPPH method in ajwa date extract, obtaining an IC50 value of 9.13 ppm and belonging to the extreme category. Meanwhile, the antioxidant activity of the anjasmoro variety soybean extract carried out by Asshidiqy *et al*. (2020) obtained an IC50 of 65.07 ppm and is included in the potent antioxidant category.

Apart from testing a single extract, mixing of the two extracts was carried out, namely at F2-F8. The highest FRAP activity was shown in a mixture of 60% date fruit extract and 40% soybean extract (F3), with an FRAP activity value of 179.882. Although the F3 mixture has a more excellent composition of date fruit extract than soybean extract, it cannot be said that this date fruit extract has a more significant role in its antioxidant activity. This can be seen from the acquisition of FRAP activity in F8 with a composition of 20% date fruit extract and 80% soybean extract, which had a higher FRAP activity value of 171.308 compared to F4, F5, and F6, which had a larger composition of date fruit extract. This is because there is a very complex interaction between each extract, so it cannot be determined which extract plays the most dominant role in providing antioxidant activity. In addition to determining antioxidant activity in single and mixed extracts, antioxidant activity was also determined in samples with a ratio of 100:100. This is done to see the antioxidant activity of the combination of samples with the same composition. Then the synergism test will be calculated.

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The synergistic effect, namely the antioxidant effect of two or more mixtures of different types of antioxidants, is greater than the effect of the individual antioxidants separately. Based on the calculations, it was found that the combination of the two extracts obtained synergistic results with a difference value of 60.273%. This percent difference value is used to determine antioxidant potential, namely having a synergistic effect if the value is positive (% difference > 0) or antagonistic if the value is negative (% difference < 0) (Skroza *et al*., 2022).

Research conducted by Vifta *et al.* (2019) showed that there was a synergistic effect on antioxidant activity using the ABTS method, which was characterized by obtaining a better IC50 from the combination of parijito fruit extract and red ginger rhizome compared to the IC50 of the single extract. Research testing the effect of synergism was previously carried out by Rani (2021) using the DPPH method on date pulp extract and raw tempeh extract. However, there is a difference in determining the effect of the synergism, namely by looking at the gain in % inhibition resulting from the combination of samples. The results of statistical tests show that the combination of samples with a ratio of 50:50 affects increasing antioxidant activity. Other synergistic effects can be determined by looking at the Combination Index (CI) value in the IC50 sample. This was implemented by Septiana *et al*. (2020), who used the DPPH method on a combination of the ethanol extract of jarong leaves and center stems. The CI value obtained was 0.7723-0.9607 and showed that the combination of the two sample extracts had a mild-to-moderate to almost additive synergistic effect. The interpretation of the CI value is <0.1, powerful synergy; 0.1-0.3 strong synergist; 0.3-0.7 synergistic; 0.7-0.9 mild–moderate synergist; 0.9-1.1 is close to additive; 1.1-1.45 mild–moderate antagonist; 1.45-3.3 antagonist; >3.3 strong–potent antagonist.

Conclusions

Date fruit extract has higher antioxidant activity than soybean extract, and combining the two has a synergistic effect. Researchers suggest that this research be continued by calculating the SPF value, testing for antibacterial activity, and conducting preclinical tests with animal mice on cosmetic preparations made from a combination of date fruit extract and soybean extract because it can produce higher antioxidant activity.

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