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The Effect of the Addition of Green Tea Extract on the Stability of Sappan Extract Due to Ultraviolet Radiation

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A B S T R A C T

This study aimed to evaluate the effect of green tea extract addition on the stability of Caesalpinia sappan (sappanwood) extract under ultraviolet (UV) radiation exposure. A completely randomized factorial design was employed using two factors: the combination ratio of sappanwood and green tea extract (100%:0%, 85%:15%, and 70%:30%) and the duration of UV exposure (0, 30, and 60 minutes). Parameters analysed included colour, antioxidant activity (IC_{50}), total anthocyanin content, and spectrophotometric absorbance. The results demonstrated that both factors significantly affected the stability of the extract. The addition of green tea extract, particularly at a ratio of 85% sappanwood to 15% green tea, yielded the highest antioxidant activity (6.77 µg/mL) and anthocyanin total (4.98%), indicating enhanced stability due to the antioxidant and photoprotective effects of catechins. However, the longer the exposure time, the lower the antioxidant activity and total anthocyanin. These findings highlight the potential of green tea as a natural copigment to enhance the photostability of brazilin-containing herbal extracts, thereby broadening their functional and pharmaceutical applications.

Contribution to Sustainable Development Goals (SDGs):
SDG 9 : Industry, Innovation and Infrastructure
SDG 13: Climate Action
SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

Indonesia, a tropical archipelago nation located between the continents of Asia and Australia, and flanked by the Indian and Pacific Oceans, harbours exceptional biodiversity. Its approximately 17,500 islands and extensive coastline of 95,181 km, coupled with a warm and humid climate and consistently high annual rainfall, create optimal ecological conditions for the proliferation of diverse plant species. It is estimated that Indonesia is home to around 40,000 plant species, of which approximately 3.2% possess bioactive compounds and are categorized as medicinal plants [1],[2],[3].

Caesalpinia sappan L., commonly known as sappanwood, is a widely recognised medicinal plant in Indonesia that has been traditionally used for its therapeutic properties. Its long-standing use is attributed to the presence of diverse phytochemical and bioactive constituents. Phytochemical investigations of C. sappan have identified various classes of phenolic compounds, including xanthones, coumarins, chalcones, flavones, and homoisoflavonoids [4]. Among these, brazilin is the predominant active compound. It is a colorless phenolic molecule characterized by two aromatic rings, a pyrone moiety, and a five-membered heterocyclic ring, which collectively contribute to its biological activity [5].

Plant-derived phenolic compounds are well-documented for their antioxidant activities, particularly in scavenging free radicals and inhibiting singlet oxygen formation [6]. Previous studies have reported the antioxidant capacity of Caesalpinia sappan extracts using various solvents. Methanolic extract of sappanwood exhibited an IC_{50} value of 9.90 µg/mL, while ethanolic extracts demonstrated values of 6.47 µg/mL [7] and 1.10 µg/mL [8]. Additionally, aqueous extract of sappanwood showed an antioxidant activity of 88.67% [9].

A compound is classified as a very potent antioxidant if it exhibits an IC_{50} value below 50 µg/mL. Compounds with IC_{50} values ranging from 50 to 100 µg/mL are considered to possess strong antioxidant activity. IC_{50} values indicate moderate activity between 100 and 150 µg/mL, while values between 150 and 200 µg/mL reflect weak antioxidant potential. Compounds with IC_{50} values exceeding 500 µg/mL are regarded as having very low or negligible antioxidant activity, although they may still hold potential as antioxidants [10].

The hydroxyl groups present in the structure of brazilin are highly susceptible to oxidation, leading to the formation of carbonyl groups [5]. Specifically, the cleavage of the hydroxyl group at C13 induces a structural transformation wherein carbonyl groups are formed at both C7 and C13. This transformation is driven by π-electron delocalization, resulting in an increased π-electron density across the molecule, which imparts a red coloration and yields brazilein as the oxidation product [11][12]. Brazilein is inherently unstable and sensitive to various environmental factors, including pH fluctuations, solvent type, and light exposure. Ultraviolet (UV) radiation, in particular, can trigger photodegradation processes [13]. To enhance the stability of brazilein, it is recommended to maintain environmental conditions such as low pH, low temperature, anaerobic atmosphere, and storage in the absence of light [14].

The aforementioned approaches represent conventional strategies to mitigate degradation processes. However, recent advancements have introduced a variety of alternative techniques to enhance compound stability. One such method is structural stabilisation through copigmentation, which has emerged as an effective strategy to enhance resistance to heat, light, and oxygen exposure [15]. Common copigments include organic acids, amino acids, phenolic compounds, and flavonoids.

Brazilin exhibits low stability, particularly when exposed to light and oxygen [16]. The incorporation of additional compounds through copigmentation has been proposed as a promising strategy to enhance the stability of this compound. In this study, green tea is selected as a copigment due to its photoprotective properties, which can increase resistance to ultraviolet-induced degradation. The antioxidant activity of green tea catechins plays a key role in mitigating oxidative damage caused by UV radiation [17]. Green tea contains a complex chemical composition with more than ten groups of bioactive compounds, notably including flavonoids particularly catechins as major constituents [18].

This study aimed to enhance the stability of brazilin, a primary compound derived from Caesalpinia sappan (sappanwood), by incorporating green tea extract as the first factor and varying the duration of ultraviolet B (UVB) light exposure as the second factor. Preliminary experiments were conducted to determine the optimal ratio of sappanwood to green tea extract, resulting in the selection of three formulations: 100%:0%, 85%:15%, and 70%:30%, respectively. The addition of green tea extract is expected to serve as an effective copigment, contributing to the improved stability of the sappanwood extract under UVB exposure.

1.2. Literature Review

1.2.1. Sappanwood

The sappan plant is a type of shrub or small tree, reaching a height of 5-10 meters, characterised by its twisted and scattered stems and branching spines. The community often uses the reddish-brown stem of sappan. When brewed, this stem produces a red color that comes from active compounds such as brazilein [19],[20]. Sappan wood is known to be rich in various chemical compounds, including flavonoids, tannins, phenolics, triterpenoids, steroids, alkaloids, and saponins, as well as unique phenolic compounds such as brazilin, xanthones, coumarins, chalcones, flavones, and homo-isoflavones [5][21].

In addition, sappan wood contains various other bioactive compounds, including phenolic acids (such as chlorogenic acid, caffeic acid, and gallic acid), anthraquinones (brazilin, brazilein, and sappanone A), and triterpenoids such as lupeol, β-amyrin, and cycloartenol [22],[23]. Other compounds, such as sappan chalcone and sappanone B are also found, which play a role in various biological activities. These ingredients make sappan wood have great potential in the pharmaceutical and medicinal fields [24].

One of the main compounds of sappan wood is brazilin, the red pigment that characterizes this wood. Brazilin, once oxidised, turns into brazilein, which has a long history as a natural dye and is used in traditional Chinese medicine to improve blood circulation, relieve menstrual pain, and act as an anti-inflammatory. Brazilin also exhibits antioxidant, anticancer, and anti-inflammatory activities. In addition, anthraquinone compounds in sappan wood play a role in pharmacological activities such as antimicrobial, anticancer, and have a laxative effect due to stimulation of colon motility[5],[21],[22],[25],[26]. Sappan chalcone and sappanone B have even been linked to the inhibition of prostate cancer cell growth and the suppression of proinflammatory cytokine production [24].

1.2.2. Green Tea

Green tea were originated from the leaves of a plant with a variety of shapes, such as radius, dissecting knife, or inverted ovoid. The leaves are approximately 30 centimetres long and 2-5 centimetres wide, with a surface that can be either downy or glossy, smooth, with jagged edges [25]. Green tea plants are found in Japan, China, and Taiwan, with the most popular type being Sencha. The chemical composition of green tea is rich, comprising polyphenols, flavonoids, phenolic acids, amino acids, proteins, fats, various essential minerals, and carbohydrates. The non-fermentation process makes green tea the best source of catechins compared to other teas[26].

Catechins are the primary flavonoid compounds in green tea, serving as antioxidants, anti-inflammatory agents, and antimicrobial agents. Types of catechins, such as EGCG, EGC, ECG, and EC, have important biological effects, including protection against urinary tract infections and the neutralisation of free radicals. Green tea also contains other compounds such as theophylline and caffeine that contribute to its health benefits. The polyphenol content in dried green tea can be as high as 30%, making this beverage an excellent source of natural antioxidants[27].

Regular consumption of green tea is known to have a positive impact on health, including reducing levels of oxidative stress compounds such as 8-hydroxyguanosine. Its antioxidant effects help prevent various chronic diseases, including lung, stomach and pancreatic cancer. Therefore, it is recommended that daily dietary intake include catechin sources, such as green tea, to support bodily functions, strengthen the immune system, and maintain overall health [28].

1.3. Research Objective

The objective of this study is to analyse the effect of green tea extract addition on the stability of Caesalpinia sappan (sappanwood) extract under ultraviolet (UV) radiation exposure.

2. MATERIALS AND METHODS

The research was conducted in January 2025 at the central instrument laboratory of the Faculty of Agricultural Technology, Universitas Andalas, in Padang city. Dried sappan wood was sourced from the traditional market in Padang city (Barkat), and green tea powder was purchased from an online store (Tea LABS).

This study employed a completely randomized factorial design, with two independent factors. The first factor was the variation in the mixing ratio of Caesalpinia sappan (sappanwood) extract and green tea extract, with sample code description :
A1 = Sappanwood extract 100%
A2 = Sappanwood extract 85% : Green tea extract 15%
A3 = Sappanwood extract 70% : Green tea extract 30%

The second factor was the duration of ultraviolet (UV) radiation exposure, with sample code description :
B1 = 0 minute exposure of UV

B2 = 30 minutes exposure of UV
B3 = 60 minutes exposure of UV

2.1 *Sappan Wood Extraction*

The dried sappan wood was ground until it passed through a 40-mesh sieve. 10 grams of sappan powder was dissolved in 100 ml of distilled water. Then, the solution was ultrasonicated for 15 minutes at 15 °C. The solution was filtered until the filtrate was obtained. Furthermore, its solvent was evaporated using a rotary evaporator [29].

2.2 *Green Tea Extraction*

Green tea was ground to a fine powder that passed through an 80-mesh sieve. Ten grams of green tea powder was dissolved in 100 ml of distilled water as the solvent. Before adding the green tea, distilled water was heated to 70 °C, and the green tea solution was stirred with a stirrer for 30 seconds. This was followed by ultrasonic treatment at 70 °C for 20 minutes. Then, the filtrate is thickened using a rotary evaporator [30].

2.3 *Radiation Of Ultraviolet Lamp*

A combination of sappan extract and green tea extract was prepared on a flat surface with a marked area of 5 x 5 cm. They were spread uniformly, then a box equipped with a UV lamp was placed. The distance between the UV lamp and the sample was set at 15 centimetres [31].

2.4 *Color Analysis*

The colour combination of sappan extract and green tea extract was visually analysed using the classic Reichs-Ausschuss für Lieferbedingungen (RAL) colour standard system by comparing the sample with the classic RAL colour standard [32].

2.5 *Antioxidant Activity*

100 mg of sample was dissolved in methanol in a 100 ml volumetric flask until it reached the mark. Furthermore, solutions with different concentrations (14, 12, 10, 8, and 6 mg/L) were prepared. Two millilitres of each concentration were placed in separate test tubes, and then 1 mL of DPPH solution was added. After that, the solution was incubated in a dark room for 15 minutes. The absorbance was measured at 517 nm.

2.6 *Anthocyanin Total*

Two sample solutions were prepared from each filtrate. In the first sample, a pH 1.0 solution was added, and for the second sample, a pH 4.5 solution was added. Then, the absorbance of each solution was measured at 510 and 700 nm [33]. The formula determined the absorbance of the reconstituted sample (A):

$$A= 〚(A_{510} - A_{700})_{pH1,0} - (A_{510} - A_{700})_{pH4,5} \quad ....(1)$$

$$\% \, Antosianin = \frac{Absorbance}{\varepsilon \, x \, L} \, x \, MW \, x \, \frac{Vd}{Wd} \, x \, \frac{1}{1000} \, x \, 100\% \quad ....(2)$$

Where :
ε = molar absorptivity of Cyanidin-3-glucoside (26,900 $L·mol^{-1}·cm^{-1}$)
L = cuvette path length = 1 cm
MW = molecular weight of Cyanidin-3-glucoside (449.2 g/mol)
Vd = final dilution volume
Wd = weight of dry extract (g)

3. RESULT AND DISCUSSION

3.1. *Yield of sappan extract and green tea extract*

After the extraction of sappan wood and green tea, sappan wood extract and green tea extract were obtained with a yield of :

Table 1. Yield of sappan extract and green tea extract

Treatment	Yield (%)
Sappan extract	2.24
Green tea extract	42.53

As shown in Table 1, the yield of sappan wood extract is 2.24%. The results are similar to those of previous research, with yields of 3.25% [29] and 3.00% [31], respectively. Various factors can affect the yield of extract compounds obtained from plant materials, including extraction methods, particle size, type and concentration of solvents, storage conditions, and the presence of interfering substances. These factors play a crucial role in determining the efficiency of extraction and the resulting yield. Nonetheless, extracts from plant materials generally still contain a diverse mixture of compounds, including various phenolic groups as well as other compounds that have solubility in the solvent used.

Sappanwood contains water-soluble flavonoid compounds, such as brazilin, protosappanin, and hematoxylin. The compound bonds in the sappan wood matrix network take time to be extracted into the solvent because breaking the bonds requires energy. In the initial stage, the energy provided will destroy the compound bonds in the solid matrix of sappan wood. After the bonds are broken, the extract compounds begin to diffuse into the solvent through the pores in the sappan wood layer, allowing for effective extraction [31].

The green tea extract yielded 42.53%, which is higher than the previous research result of 28.9% [32]. A high yield indicates that more compounds are dissolved in the solvent. One of the factors that affect the yield of an extraction is the solubility of the solvent used. Water is classified as a solvent with a high level of solubility, which enables the production of a high yield [32]. Green tea contains various polar compounds, including polyphenol groups, alkaloids, and amino acids. Polyphenols in green tea account for 20-30% of the total compound content, with catechins being the main component. Catechins in green tea include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG)[33].

3.2. *Color Analysis*

The color produced by sappanwood extract is influenced by the solvent used. In the water extract of sappanwood, the color produced is pearl ruby red, in the ethanol extract of sappanwood, the color produced is between pearl ruby red and traffic red, and in the propanol extract of secang wood, the color produced is traffic red [29]. The result is shown in Table 2.

3.3. *Antioxidant Activity*

The results showed that the higher concentration of green tea extract was effective in reducing the antioxidant activity of the combination extract. The highest antioxidant activity was observed in the combination of sappan extract and green tea extract with a ratio of 85%:15%, which was 6.77 µg/mL. This result is higher compared to previous research, which reported values of 11.46 µg/mL and 11.42 µg/mL, respectively [34][35].

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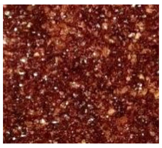
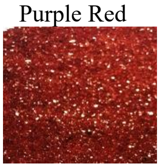
Table 2. Color Result

Combination Factors	Color – Duration Time Factors: 0 min	30 mins	60 mins
(100% : 0%)			
	Ruby Red	Pearl Ruby Red	Pearl Ruby Red
(85% : 15%)			
	Purple Red	Wine Red	Oxide Red
(70% : 30%)			
	Purple Red	Wine Red	Black Red

Description : Color result base on standard color Reichs-Ausschuss für Lieferbedingungen (RAL).

Table 3. The Effect of Ratio on Antioxidant Activity

Treatment	Antioxidant activity (µg/mL)
(100% : 0%)	6.88 a
(85% : 15%)	6.77 a
(70% : 30%)	8.70 b

*5% significance level of DNMRT

As shown in Table 3, the variation in the ratio between sappan wood extract and green tea extract has a significant influence on antioxidant activity ($p < 0.05$).

Table 4. The Effect of Duration on Antioxidant Activity

Treatment	Antioxidant activity (µg/mL)
(0 minute)	6.24 a
(30 minutes)	7.31 b
(60 minutes)	8.80 c

*5% significance level of DNMRT

As presented in Table 4, ultraviolet (UV) exposure time significantly affects antioxidant activity ($p < 0.05$), whereby extended exposure durations are associated with reduced antioxidant activity. The longer exposure time and the closer exposure distance to UV radiation result in lower antioxidant activity, which is similar to previous research [36].

3.4. *Anthocyanin Total*

This study has an anthocyanin total average of around 4.76% to 54.85%, which is higher compared to previous research with 2.43% [37]. For the detail shown in Table 5 and Table 6.

Table 5. The Effect of Ratio on Anthocyanin Total

Treatment	Anthocyanin Total (%)
(100% : 0%)	4.82 a
(85% : 15%)	4.98 a
(70% : 30%)	4.85 b

*5% significance level of DNMRT

As presented in Table 5, variations in the ratio between sappan wood extract and green tea extract significantly influence total anthocyanin content ($p < 0.05$).

Table 6. The Effect of Duration on Anthocyanin Total

Treatment	Anthocyanin Total (%)
(0 minute)	5.02 b
(30 minutes)	4.87 a
(60 minutes)	4.76 a

*5% significance level of DNMRT

As shown in Table 6, ultraviolet (UV) exposure time significantly affects total anthocyanin content ($p < 0.05$), with extended exposure durations resulting in a marked reduction in anthocyanin levels.

Anthocyanins are water-soluble natural pigments that are found in flowers, fruits, and leaves of various plants. This pigment produces orange, red, and blue colors, depending on the pH of the environment. Apart from providing bright colours, anthocyanins are also known to possess antioxidant properties that are important for health, one of which is utilised in various cosmetic products, such as lotions and creams [38].

4. CONCLUSION

The addition of green tea extract significantly enhanced the stability of Caesalpinia sappan (sappanwood) extract under ultraviolet (UV) radiation exposure. This improvement is attributed to the photoprotective and antioxidant properties of catechins present in green tea, which helped mitigate the degradation effects caused by UV radiation. This was evidenced by the highest antioxidant activity observed at a mixing ratio of 85% sappanwood extract to 15% green tea extract, reaching 6.77 µg/mL and a total anthocyanin content of 4.98%. However, the longer duration of UV exposure decreases antioxidant activity and total anthocyanin.

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