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Caesalpinia Sappan: Antioxidant, Anticancer, and Related Properties of Extracts in Cell Lines. A Literature Review

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ABSTRACT : Sappan wood (Caesalpinia sappan L.) has been known to have various healing benefits and is often used by the community as a health drink. The methodology used in this research involves searching scientific literature using search engines. The leaves of sappan wood contain polyphenol compounds and have an essential oil content of 0.16-0.20%. sappan wood have antioxidant activity, including flavonoids, alkaloids, polyphenols, terpenoids, triterpenoids, tannins, and saponins. The anticancer activity of sappan wood, as reviewed in several studies, shows that the brazilin and brazilein compounds contained in sappan wood have the ability to inhibit the growth of cancer cells in some cell cultures.

Keywords – Antioxidant, Anticancer, Sappan wood

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1. INTRODUCTION

People have used herbal medicine since ancient times to treat various health problems. More than half a million of plant species on Earth provide rich sources of phytochemicals that have therapeutic potential. Secondary metabolites produced by plants serve as a protection against pathogens and disadvantageous environmental conditions.

Indonesia has a rich biodiversity. The diverse kinds of flora in Indonesia can be used as sources of chemical compounds that have various types and quantities. These chemical compounds can be used as cosmetics, insecticides, medicines, and raw materials for synthesizing other organic compounds. Many types of plants in Indonesia can be used as herbal medicines, one of which is the sappan wood plant (Caesalpinia sappan L.).

Sappan wood (Caesalpinia sappan L.) is a plant from the Caesalpiniaceae family that is widely distributed in Indonesia. Traditionally, sappan wood has been known to have various healing benefits and is often used by the community as a health drink. The leaves of sappan wood contain polyphenol compounds and have an essential oil content of 0.16-0.20%. The branches of the sappan wood plant contain several compounds, including phenolic acids, polyphenols, and flavonoids [1].

Sappan wood is a plant that contains gallic acid, brazilin (red sappan dye), and tannic acid. Several triterpenoids, flavonoids, and oxygen heterocycles have been found in the isolated compounds from sappan wood, and brazilin has been found as the main component, which is believed to play a significant role in the pharmacological effects of sappan wood. Brazilin is an antioxidant compound with the chemical structure C16H14O5. It has various pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant, antiviral, and

anticomplementary effects. It is a key component and distinguishing compound of sappan wood and due to its antioxidant activity, brazilin can protect the body from free radical toxicity [2]. Free radicals are molecules that have unpaired electrons in their outer shell, therefore, they are unstable and highly reactive. These molecules always seek other electrons to bond with, which react with other substances in the body and form additional free radicals that can be harmful to humans [3]. If the exposure continues to happen, free radicals can overwhelm the body's antioxidant capacity and lead to an imbalance known as oxidative stress. As a result, certain molecules can be oxidized, which may cause carcinogenic effects, weaken the immune system, accelerate aging, and lead to various diseases.

Free radicals are created naturally from the body's metabolic processes, such as cellular metabolism, phagocytosis, arachidonic acid metabolism, ovulation, and fertilization. These substances can damage lipids, proteins or enzymes, carbohydrates, and DNA in cells or tissues, that can lead to membrane damage, protein modification, DNA damage, and cell death [4]. However, free radicals can be neutralized with antioxidants [5].

Research by [6] shows that water extract from sappan wood has higher antioxidant activity compared to commercial antioxidants such as Butylated Hydroxytoluene (BHT) and Tertiary Butyl Hydroquinone (TBHQ), which makes sappan wood a potential agent to ward off free radicals. BHT and TBHQ are synthetic compounds with high antioxidant activity similar to natural vitamin E [7].

Over the past three decades, the preference in using natural products for treating diseases has increased significantly. Plant-based medicine, which has long been practiced traditionally in various cultures, is recognized as highly effective to treat many conditions that are difficult to manage with allopathic systems. Many studies have been conducted to explore the beneficial effects of plant extracts in treating diseases. This systematic review aims to synthesize the results and conclusions from validated studies on the antioxidant, anticancer, anti-inflammatory, and other related effects of sappan wood extract. This study will consolidate current evidence on the impact of sappan wood extract in living cell cultures. There is a special focus on the objective conclusions derived from laboratory techniques such as cell proliferation assays, cytotoxicity tests, oxidative stress assays, PCR, and analysis of genes related to inflammation and cancer.

2. METHOD

The methodology used in this research involves searching scientific literature using search engines such as Google Scholar, Science Direct, Elsevier, and PubMed. Keywords used in searching the literature include "sappan wood," "phytochemistry," "antioxidant," and "anticancer." The authors focused on both national and international journals, as well as other relevant literature that support the topic. After gathering the literature, the data were combined and thoroughly reviewed to provide a comprehensive information that describe the phytochemical content, antioxidant activity, and anticancer properties of sappan wood. The end goal of this research is to develop a potential review of sappan wood extract.

3. DISCUSSION

Sappan wood was first discovered in Brazil by a Spanish named Kimichi, and the plant is known as ‘Brazil wood’ due to its origin. However, some believe that the plant originally came from India and spread through Burma, Thailand, Indochina, Malaysia, and later to Indonesia, the Philippines, Sri Lanka, Taiwan, and Hawaii. Sappan wood thrives in various regions like Europe, the America, and Asia. The plant's scientific name is Caesalpinia sappan, and it is also known by the synonym Bianceae. In many countries, this plant is known with various names, such as: 'sibukao' in the Philippines, 'teingnyet' in Burma, 'sbaeng' in Cambodia, 'fang deeng' in Laos, and 'faang' in Thailand. In Indonesia, sappan wood is known by a range of local names depending on the region, including: seupeng (Aceh); sepang (Gayo); sopang (Batak);n cacang (Minangkabau); secang (Sunda); kayu secang, soga Jawa (Jawa); kaju secang (Madura); cang (Bali); sepang (Sasak); supa, suang (Bima); sepel (Timor); ; hong (Alor); kayu sema (Manado); dolo ; sapang (Makassar); seppang (Bugis); sefen (Halmahera Selatan); sawala, hiniaga, sinyiang, singiang (Halmahera Utara); sunyiha (Ternate); dan roro (Tidore) [8]

The classification of sappan wood can be seen as follows [9], [10]:

Regnum : Plantae
Division : Spermatophyta
Sub division : Angiospermae
Class : Dicotyledoneae
Ordo : Rosales
Family : Caesalpiniaceae
Genus : Caesalpinia
Species : *Caesalpinia sappan* L

Phytochemical Test of SappanWood

Table 1. Some observational studies of sappan wood phytochemical test

Source	Phytochemical Test of SappanWood Result
[11]	Sappan wood's ethanol extract contains flavonoid glycosides, free flavonoids, alkaloids, and polyphenols
[11]	Sappan wood's ethanol extract contains flavonoid glycosides, alkaloids, flavonoid glycosides and polyphenols
[12]	Sappan wood's ethanol extract contains terpenoids, flavonoids, and phenolics
[13]	Sappan wood's ethanol extract contains tannins, phenolics, flavonoids, and triterpenoids
[14]	Sappan wood's ethanol extract contains phenolic components with various types of structure, including campesterol, coumarin, xanthone, chalcones, homoisoflavonoids, flavones, and brazilin
[15]	Ethanol extract contains: Alkaloids, terpenoids, tannins, flavonoids, steroids, and phenolic Water extract contains: Alkaloids, terpenoids, tannins, and phenols Methanol extract contains: Saponins
[16]	Ethanol extract contains homoisoflavonoids, chalcones, dibenzoxocins, and brazilins
[17]	flavonoids, saponins, polyphenols, and essential oils
[18]	Methanol extract contains: steroids, phenolics, terpenoids, and anthraquinones

A research conducted by Prahasti and Hidajati [19] using sappan wood's ethanol extract showed positive results in the test of alkaloid compounds using Dragendorf reagent, flavonoids with shinoda test, phenolics, tannins, and triterpenoids. The qualitative test for tannin compounds showed a brownish-green color, that means the ethanol extract of sappan wood is included in the tannin compounds. Meanwhile, in phenolic compounds testing, the ethanol extract showed a positive result, that was indicated by the black-brown color formation of the $[Fe(OAr)6]^{3-}$ complex.

A research by Muthiah et al. [20] using thick extract and dry extract of sappan wood's bark showed a positive phytochemical test result containing secondary metabolites of saponins, tannins, polyphenols, steroids, and flavanoids. In addition, it is also found that the best phenolic content was obtained in dry extract of sappan wood with the addition of lactose filler of (513,70 ± 44,52) mg EAG /g extract.

Antioxidant Activity of Sappan Wood

Table 2. Antioxidant activity of sappan wood reviewed from several literatures

Source	Method	Testing Results
[11]	DPPH ABTS FRAP	Sappan wood has strong antioxidant compounds with IC50 values of DPPH: 101.47 ppm, ABTS: 26.70 ppm, and FRTP: 11.37 ppm
[21]	DPPH	The optimal conditions for extracting sappan wood with 65% ethanol solvent are at a temperature of 30°C for 40 minutes, with antioxidant concentrations ranging from 1.0577 ± 0.0019 to 4.2759 ± 0.0017 mg/mL
[22]	RAL	Antioxidant activity in sappan wood is 85.58%
[23]	DPPH	Antioxidant extract obtained by heating water to ±95°C for 30 minutes shows an antioxidant value of 1601.7 ppm
[19]	Maserasi	The antioxidant activity value for sappan wood is 164.782 ppm
[24]	DPPH	Antioxidant activity with the DPPH method gives an IC50 value of methanol extract: 1.75 ppm and Ethyl Acetate Fraction: 0.88 ppm
[3]	KLT-DPPH	Antioxidant compounds that were successfully isolated include alpinetin with IC50 values: 20,11 µM and a 3-deoxysappanone B compund with an IC50 value of: 15,28 µM
[25]	DPPH	The IC50 value of sappan wood's ethanol extract is 56.32 µg/mL, which classifies as a very strong antioxidant. This is indicated by the presence of flavonoids with an orange color

A research by [26], titled "Characterization and Study of Antioxidant Activity of Ethanol Extract of Sappanwood," focused on the sappan wood plant in the Solo area of Central Java. The antioxidant activity of the ethanol extract of sappanwood was determined using the Ferric Reducing Antioxidant Power (FRAP) method, resulting the amount of 13.99 mmol Fe(II)/100 g. Based on the results obtained, after being compared with the antioxidant activity of gallic acid, the antioxidant activity of the sappan wood extract is higher (9.34 mmol Fe(II)/100 g). The primary component of sappanwood is brazilin, which has more hydroxyl groups compared to gallic acid. This

structural feature likely contributes to the superior antioxidant activity of brazilin.The greater the number of hydroxyl groups in a sample, the higher the antioxidant activity, as these groups can donate H atoms to free radicals and pair them, which makes them less reactive. The ethanol extract of sappan wood also has better antioxidant activity than other antioxidants, such as BHT and TBHQ.

A research conducted by [11] used ethanol extract of sappan wood (*Sappan Lignum*) from the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) in Tawangmangu. The antioxidant activity testing, conducted using the DPPH, ABTS, and FRAP methods, demonstrated that the sappan wood's ethanol extract contained strong antioxidant activity. Blois [27] stated that a compound is considered a very strong antioxidant if it has an IC50 value of less than 50 ppm. This can be seen from the IC50 values of each test and can also be seen by comparing the IC50 values with trolox. Trolox is a water-soluble antioxidant, which is synthesized as a derivative of Vitamin E in 1974. It is also commonly used as a comparison standard reference in various antioxidant tests.

Anticancer Activity of Sappan Wood

Table 2. Anticancer activity of sappan wood reviewed from several literatures

Source	Testing Results
[28]	Sappan wood's ethanol extract has the ability to inhibit the growth of cancer cells by inducing apoptosis and mitochondrial dysfunction in A549 cells
[29]	The brazilin compound found in sappan wood extract has anticancer activity by inhibiting the apoptosis-inhibiting protein surviving
[30]	The brazilein content in sappan wood is cytotoxic to T47D cells and can enhance the cytotoxic effect of cisplatin by inducing apoptosis
[31]	Sappan wood extract with various polar solvents, has cytotoxic activity and inhibits matrix metalloproteinase in 4T1 breast cancer cells
[32]	Brazilin contained in sappan wood has cytotoxic effects on cells such as MCF-7, 4T1, and T47D cell cultures, one of which is by inhibiting HO-1 expression induced by heme through inactivation of the JNK/Nrf2 pathway in MCF-7 cells

One method to assess the anticancer potential of natural substances is by observing the inhibition of growth in test cells. A research by [33] used methanol extracts to examine the anti-proliferative effects in-vitro on several cancer cell lines such as HeLa, HT-1080, A549, 26-L5, LLC, and B16-BL6. The research proved that the extract could inhibit the proliferation of HT-1080, HeLa, and LLC cells at low concentrations. Another method that can be used to evaluate anticancer activity is by assessing the cytotoxic effects of the substance on the test cells.

Mar et al. [34] investigated the anticancer potential of sappan wood's methanol extract using the DNA-strand scission method. The experiment showed an IC50 value of 5.9 µg/ml, where the active component responsible for the DNA strand-scission activity is brazilin. An in-silico study of the brazilin molecule was conducted to explore its ability to activate Adenosine Monophosphate Activated Kinase (AMPK) and an in-vitro study to test the anticancer potential of methanol extract from Caesalpinia sappan Linn wood bark on the MCF-7 breast adenocarcinoma cell line. The MeOH extract of Caesalpinia sappan Linn showed potential inhibitory activity against proliferation and induced apoptosis in MCF-7 cells. The docking interaction score between brazilin and AMPK suggests that brazilin is a promising AMPK activator candidate with anticancer properties [35]

4. CONCLUSION

Based on the literature review conducted, it can be concluded that the compounds in sappan wood (Caesalpinia sappan L.) have antioxidant activity, including flavonoids, alkaloids, polyphenols, terpenoids, triterpenoids, tannins, and saponins. The antioxidant activity of sappan wood has been tested using various methods. The anticancer activity of sappan wood, as reviewed in several studies, shows that the brazilin and brazilein compounds contained in sappan wood have the ability to inhibit the growth of cancer cells in some cell cultures.

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