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# Exploring Green Food Packaging Properties of *Bauhinia variegata*, *Prunus dulcis*, *Tectona grandis* and *Nelumbo nucifera* – A Comparative Analysis

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## Abstract

**Objectives:** The purpose of this current research is to develop suitable food packaging films with extended shelf using medicinal plant leaves. **Methods:** The four medicinal plants for their traditional food packaging leaves, namely Mantharai (*Bauhinia variegata*), Badam (*Prunus dulcis*), Teak (*Tectona grandis*), and Lotus (*Nelumbo nucifera*) were used. Qualitative determination of the phytochemical compounds in the aqueous extracts of all four leaves were done. The antioxidant activities were assessed by DPPH Radical Scavenging assay and Ferric Reducing Antioxidant Power assay. The antibacterial and antifungal activity was screened against the bacterial (*Staphylococcus aureus*, *Escherichia coli*, and fungal species *Candida albicans*, *Aspergillus flavus* of clinical importance using the disc diffusion method. **Findings:** *B. variegata* showed the highest flavonoid content of 2.273 $\pm$ 0.0021 mg Catechin Equivalent /100 mg and presented potent antioxidant activity in DPPH and FRAP assays. Among all the extracts screened, *N. nucifera* and *T. grandis* had the highest phenolic content of (886.5 $\pm$ 0.776 $\mu$g GAE/100 mg and 861.5 $\pm$ 1.211$\mu$g Gallic Acid Equivalent/100 mg respectively). The antimicrobial activity indicated high efficacy of *B. variegata* (17 mm against *S. aureus* at 500 $\mu$g/ml), followed by *N. nucifera* with reasonable inhibition zones (13 mm against *C. albicans* and 12 mm against *E.coli* both at 500 $\mu$g/ml. The other extracts had moderate to weak antioxidant and antimicrobial activity compared to the standard positive control. From the percentage inhibition of antibacterial activity, it was revealed that the extract of *B. variegata* was able to inhibit the growth of *S. aureus* up to 94.44 % and *E. coli* up to 94.12 % at the concentration of 500 $\mu$g/mL compared to Ciprofloxacin (100 %). Similarly, *N. nucifera*, *P. dulcis*, and *T. grandis* extracts displayed inhibition ranging from 0 % at 100 $\mu$g/mL to 84. 21%, 66. 67% and 35% at 500 $\mu$g/mL. Inhibition of *C. albicans* by *B. variegata* was recorded to be 94.12% while it was 60% for *A. flavus. N. nucifera* maceration reached 85.71 % inhibition of *C. albicans* and 47.06% for *A. flavus*. With *P. dulcis* and *T.*

*grandis* extracts at a concentration of 500 $\mu$g/mL, the inhibition was recorded to be 63.16% and 70% respectively to that of fluconazole (100%). **Novelty:** This study explores the usage of *Bauhinia variegata* as eco-friendly green food packaging material. It is evidenced that due to their highly efficient antioxidant and antimicrobial characteristics, these natural polymers present in *B. variegata* might overcome oxidative stress and microbial harm in food and packaging products for sustainable and safe applications.

**Keywords:** Phytochemical analysis; Antimicrobial activity; Antioxidant activity; Bauhinia variegata Linn; Food packaging

## 1 Introduction

Growing demand globally for the development of sustainable and environmentally friendly green food packaging has led to increased attention to natural bioactive materials. Traditionally, food packaging materials relied greatly on plastics and synthetic additives were profoundly used for their durability, preservation capabilities, cost-effectiveness and their functional attributes. However, the major disadvantages of these plastics are non- biodegradability and often pose environmental disquiets and health hazards due to their toxic nature. This context emphasizes the need for innovative solutions for food packaging with integral bioactive characteristics for sustainable and safe for consumers.

*Bauhinia variegata Linn*. has been examined for its various biological functions. The plant's parts like leaves and bark have strong antioxidant attributes due to flavonoids, tannins, and phenolic compounds. Research displays antimicrobial effects against bacteria such as *Escherichia coli* and *Staphylococcus aureus*. Investigations using human cancer cell lines reveal notable anti-cancer effects of *Bauhinia variegata Linn*. extracts. Additionally, it demonstrates anti-inflammatory effects by inhibiting pro-inflammatory cytokines and enzymes[1,2].

*Prunus dulcis L*. is recognized for its important biological activities, especially its strong antioxidant properties associated with its high phenolic levels. These compounds efficiently neutralize free radicals, lowering oxidative stress in cells. Extracts from this tree also show anti-inflammatory properties that help relieve arthritis and asthma symptoms. In addition, it exhibits broad-spectrum antimicrobial action against several pathogens and supports liver health by reducing liver enzyme levels[3,4].

The therapeutic properties of all parts of the teak tree (infusion of bark for skin diseases, leprosy, bronchitis, hyperacidity, dysentery and verminosis, burning sensations, diabetes and obstructed labour[5] are a traditional cure to possible adverse effects associated with the medicinal use. Teak leaves are useful for stopping bleeding of wounds and have hemostatic properties, and are very effective for inflammatory diseases[6]. A recent article discussed the astringent property, topical or oral application of leaf extracts helps in wound healing by increasing breaking strength as well as collagen formation[7].

The sacred lotus or Indian lotus, *Nelumbo nucifera*, is the sole species of the monocot family Nymphaeaceae. We selected this plant because of its various biological activities; due to the large amount of flavonoids found in leaf extract and seed decorating, potently scavenges free radicals against oxidative stress. In vitro, bioactive compounds in the seedpod and leaves, such as quercetin, inhibit cancer cell growth. Lotus leaf extract has exhibited high antimicrobial activity against a range of pathogens, including multidrug-resistant bacteria and fungi. In addition to the former, *Nelumbo nucifera* showed neuroprotective activity against neurons exposed to oxidative stress and those containing neutrophils may also prevent the occurrence of degenerative diseases such as Alzheimer's disease.[8,9]

Although earlier research has documented various applications of *B. variegata*, *Prunus dulcis*, *T. grandis* and *N. nucifera* in pharmaceuticals, their use in food packaging has not been widely investigated. This study is among the few[10,11] that aim to compare the biological activities of these plants, particularly in green packaging. This research seeks to establish the level of phytochemicals, antioxidant capacity as well as antimicrobial activities of the extracts of those leaves so as to determine which of the plant extracts has high potential for use in packaging.

## 2 Methodology

### 2.1 Collection and extraction process of the leaves of four selected species of plants

In the present study, the leaves of *B. variegata* (Mantharai), *P. dulcis* (Badam), *T. grandis* (Teak) and *N. nucifera* (Lotus) were collected from various parts of Chennai, Tamil Nadu, India. After collection, the leaves were washed thoroughly with $ddH_2O$ and shade-dried. After that, the dried leaves were powdered and subjected to the extraction process. 10 gm of dried leaves powder was soaked in 100ml of $ddH_2O$ and kept for 72 hr at RT for extraction. After 72 hrs, the obtained aqueous extract was filtered through Whatmann no.1 paper and evaporated using a rotatory evaporator. The final concentrate of extract was stored at 4°C until further analysis.

### 2.2 Preliminary qualitative phytochemical analysis of aqueous extract of leaves

The presence of various secondary metabolites in the aqueous extracts was carried out by following the standard methodology[12].

### 2.3 Quantitative analysis of Total Phenols, Flavonoids, and Alkaloids of aqueous extract of leaves

The total Phenolic contents were analysed using the folin-ciocalteu reagent method,[12] total flavonoid content (TFC) was carried out by using aluminium chloride by UV-spectrophotometric method,[12] and the total alkaloid content was quantified based on Bromocresol Green complex method[13].

### 2.4 Total in vitro Antioxidant activities of aqueous extract of leaves

The antioxidant activity of the plant extracts was determined using DPPH radical scavenging method and the FRAP method. In DPPH assay, the antioxidant reactivity was determined by reduction of the stable free radical DPPH, where the antioxidants donate, an electron or hydrogen ion. For the assay, 1 mL of 0.1 mM solution of DPPH in methanol was reacted with 1 mL of varying concentrations of the plant extract by allowing the mixture to stand in the dark for half an hour before the absorbance value was read on a spectrophotometer at 517 nm. The percentage inhibition was determined through the equation % Inhibition = [(A control - A sample)/A control] $\times$100. The ability of the extracts to reduce ferric ions ($Fe^{3+}$) into ferrous ions ($Fe^{2+}$) was examined and the blue complex with TPTZ formed was measured. FRAP reagent was made from 300mM acetate buffer at pH 3.6, 10mM TPTZ in 40mM Hcl and 20mM ferric chloride. To 3 ml reagent, 100 $\mu$l of the plant extract was added and the reaction mixture was allowed to incubate at 37°C for 30 minutes. Absorbance was processed at 593 nm and contents were presented as $\mu$mol $FeSO_4$ equivalent per gram of extract as a sign of antioxidant activity[14].

### 2.5 Antimicrobial analysis of aqueous extract

The antimicrobial activity of the plant leaf extracts was evaluated using the disc diffusion method. Sterile filter paper discs were impregnated with aqueous extract solutions at concentrations of 100 $\mu$g, 200 $\mu$g, 300 $\mu$g, 400 $\mu$g, and 500 $\mu$g per disc and placed on the surface of agar plates pre-inoculated with the test microorganisms. Mueller-Hinton agar was used for bacterial strains, while Sabouraud dextrose agar was used for fungal strains. Ciprofloxacin (10 $\mu$g/disc) and fluconazole (10 $\mu$g/disc) served as positive controls for bacteria and fungi, respectively. The plates were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 48 hours for fungal strains. After incubation, the zones of inhibition around each disc were measured in millimeters to determine the antimicrobial efficacy of the extracts[15].

### 2.6 Statistical analysis

The results from the experiments were presented as the mean $\pm$ standard deviation (SD).

## 3 Results and Discussion

From the results obtained, as shown in Table 1 the phytochemical screening of aqueous extracts of four medicinal plants revealed the presence of phenols, alkaloids, tannins, flavonoids and saponins. However, *B. variegata* showed the presence of alkaloids, phenols, flavonoids and carbohydrates. Generally, these preliminary results suggest the presence of medicinal properties that could correlate with antioxidant activities and other biological properties.

**Table 1. Qualitative Phytochemical analysis of aqueous extracts*Bauhinia variegata* (Mantharai), *Prunus dulcis*(Badam), *Tectona grandis* (Teak), and *Nelumbo nucifera* (Lotus)**

| S.no | Phytochemical tests | *Bauhinia variegata* ( mantharai) | *Prunus dulcis* (badam) | *Tectona grandis* (Teak) | *Nelumbo nucifera* leaves extract (lotus) |
|---|---|---|---|---|---|
| **1** | Wagner's tests | + | + | + | + |
| **2** | Ninhydrin test | + | - | - | + |
| **3** | Benedict's tests | >2% | 0.50% | 2% | 2% |
| **4** | Fehling's test | >2% | 2% | 2% | 0.50% |
| **5** | Alkaline reagent test | + | + | + | + |
| **6** | Foam test | + | + | - | + |
| **7** | Copper acetate test | - | - | - | - |
| **8** | Salkowski test | + | + | + | - |
| **9** | Picric acid test | + | + | + | - |
| **10** | Gelatin test | - | + | + | - |
| **11** | Ferric chloride test | + | + | + | + |
| **12** | Labat test | + | - | + | + |

**(+) – Positive ; (-) – Negative**

Table 2 reveals the total phenolic, flavonoid and alkaloid content of the aqueous extract of the leaves of the plant. The total phenolic content at value ranged between 786.22 $\pm$ 1.814, 886.5 $\pm$ 0.776 and 861.5 $\pm$ 1.211$\mu$g GAE/100 mg of extracts was recorded with N. nucifera, *T. grandis* and *P. dulcis* respectively. *T. grandis* with 861.5 $\pm$ 1.211 $\mu$g GAE/100 mg, and *P. dulcis* at 786.22 $\pm$ 1.814$\mu$g GAE/100 mg. As for the phenolic content of *B. variegata* was found to contain the lowest amount of phenolic content with 1.097 $\pm$ 0.004$\mu$g GAE/100 mg. The results suggest that *N. nucifera* and *T. grandis* contain high levels of phenolics which are known vitamins and antioxidants. The results of the flavonoid content revealed that the highest flavonoid content was observed in *B. variegata* 2.273 $\pm$ 0.0021 mg CE/ 100 mg and therefore has the prospect as a source of flavonoids since they help in anti-inflammatory and antioxidant activity. The flavonoid level of *T. grandis* was 2.017 $\pm$ 0.002 mg CE/100 mg and *P. dulcis* was 1.97 $\pm$ 0.008 mg CE/100 mg but the flavonoid content of *N. nucifera* was the lowest, only 1.938 $\pm$ 0.001 mg CE/100 mg.

**Table 2. Quantification of Total Phenolic, Flavonoids and Alkaloids content of aqueous extract of leaves**

| | Total Phenolic content ($\mu$g GAE/100mg) | Total Flavonoids content (mg of CE/100mg) | Total Alkaloids content ($\mu$g/100 mg ) |
|---|---|---|---|
| *Bauhinia variegata* (Mantharai) leaves extract | 1.097$\pm$0.004 | 2.273$\pm$0.0021 | 2.161474$\pm$0.0118 |
| *Nelumbo nucifera* (Lotus) leaves extract | 886.5$\pm$0.776 | 1.938$\pm$0.001 | 1.604423$\pm$0.105 |
| *Tectona grandis* (Teak) leaves extract | 861.5$\pm$1.211 | 2.017$\pm$0.002 | 1.3025$\pm$0.024 |
| *Prunus dulcis* (Indian Badam) leaves extract | 786.22$\pm$1.814 | 1.97$\pm$0.008 | 1.229423$\pm$0.0126 |

N=3 ; ($\pm$) Represents SD value

The results of the antioxidant assays analysed by DPPH and FRAP demonstrated their potent antioxidant efficacy through scavenging free radicals and reducing ferric ions. When comparing all concentrations of the radical scavenging activity of the extracts in the DPPH assay (Figure 1(**a**)), it was identified that L-Ascorbic acid proved to have the highest activity while among the plant extract; *Bauhinia variegata* and *N. nucifera* had significantly high activity. On the other hand, *T. grandis* and *P. dulcis* had moderate to low activities in antibacterial properties. Also, in the FRAP assay, as illustrated in L-Ascorbic acid had higher ferric reducing power than the extracts studied here as seen in Figure 1(**b**). The two assays showed a dose-response pattern where the antioxidant activity of the extracts increased with an increase in extract concentration which implies that radicals scavenging and Fe(III) reduction are directly proportional to extract concentration. Taken together, the findings suggest that *N. nucifera* and *B. variegata* exhibit measurable antioxidant potentials that may have therapeutic value for further investigation.

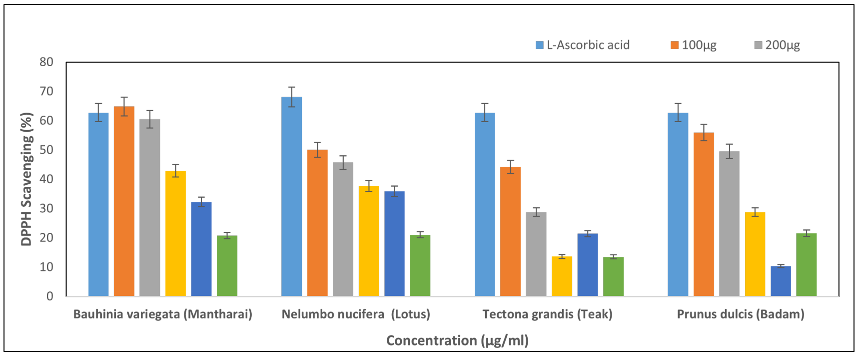


**Fig 1. DPPH assay**

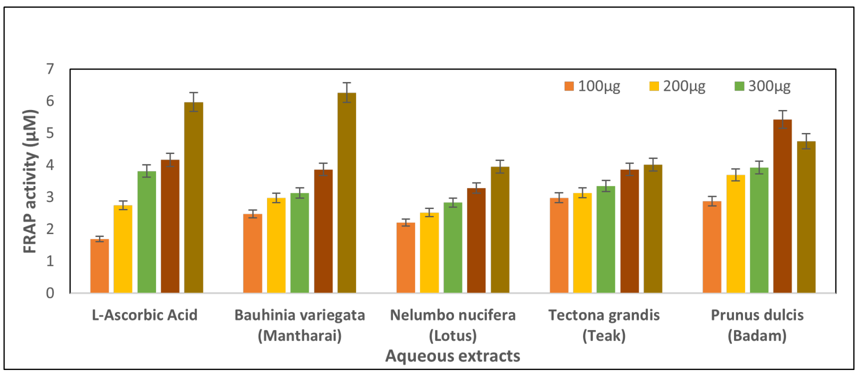


**Fig 2. FRAP - Activity**

The inhibition zone diameter and the percentage of antimicrobial activity showed that the aqueous leaf extracts from different plant species exhibited significant variations in the examined microbial strains. Among the plants studied, inhibition against *Staphylococcus aureus* was significant with Mantharai (*B. variegata*) which rose from a zone of inhibition of 8 mm at the concentration of 200 $\mu$g/mL to 17 mm at the concentration of 500 $\mu$g/mL. It also showed a moderate zone of inhibition against *Escherichia coli*; 16 mm at 500 $\mu$g/ml and against *C. albicans*; 16 mm at 500 $\mu$g/ml. However, there was no significant inhibition against *A. flavus*.

*P. dulcis* (Indian Badam) was found moderately effective against *Staphylococcus aureus* with a maximum zone of inhibition of 12mm at 500 $\mu$g/ml was observed whereas no zone of inhibition was recorded against *Escherichia coli*. Its activity against *C. albicans* augmented to 11 mm at higher concentrations of the extract (500 $\mu$g/ml) and moderate inhibition (8 mm) was noted for *A. flavus*. Lotus on the other hand was able to show a concentration dependant antimicrobial activity, which was very much pronounced against *C. albicans* and *E. coli* with a zone of inhibition of 13 mm and 12 mm, respectively, at a concentration of 500 $\mu$g/ml. A good level of inhibition occurred against *Staphylococcus aureus* with an IZD of 8 mm at 300 $\mu$g/ml and very poor inhibition against *A. flavus*. The result revealed that *T. grandis* (Teak) had moderate antimicrobial activities against the selected strains. The extract exhibited inhibition zones of 7mm against both *S. aureus* and *E. coli* at 500 $\mu$g/ml and *C. albicans* at 500 $\mu$g/ml. While *A. flavus* was not tested, no inhibition was observed, at 100 $\mu$g/ml and 200 $\mu$g/ml.

Other studies on the various extracts of *B. variegata* demonstrated the anticancer activity of *B. variegata*, particularly the secondary metabolites. The entire plant and its various organs were extracted using solvents of different polarity to obtain different phytochemicals that were analyzed, assayed for antioxidants, and evaluated for their biological activities. The present study reveals that methanolic extracts contain high phenolic and flavonoid contents, strong cytotoxicity, and antiproliferative activity against MFL7 breast cancer cells and good activity against both Gram-positive and Gram-negative bacterial strains, and significant antidiabetic activity [16,17].

**Table 3. Antibacterial activity of aqueous extracts**

| | | *Staphylococcus aureus* | | | | *E.coli* | | | |
|---|---|---|---|---|---|---|---|---|---|
| **S. No** | **Concentration of Aqueous Extract ($\mu$g/mL)** | **(*B. variegata*)** | **(*N. nucifera*)** | **(*P.dulcis*)** | **(*T. grandis*)** | **(*B. variegata*)** | **(*N. nucifera*)** | **(*P. dulcis* )** | **(*T. grandis*)** |
| 1. | Positive control (Ciprofloxacin 10 $\mu$g/mL | 18mm | 19mm | 18mm | 20mm | 17mm | 16mm | 19mm | 18mm |
| 2 | 100 ($\mu$g/mL) | NIL | NIL | 6 mm | NIL | NIL | NIL | 6 mm | NIL |
| 3 | 200($\mu$g/mL) | 9 mm | 9 mm | 8 mm | NIL | 9 mm | 7 mm | 8 mm | NIL |
| 4 | 300($\mu$g/mL) | 11 mm | 12 mm | 10 mm | 6mm | 12 mm | 10 mm | 10 mm | 6mm |
| 5 | 400 ($\mu$g/mL) | 13 mm | 14 mm | 11 mm | 7mm | 14 mm | 11 mm | 11 mm | 7mm |
| 6 | 500 ($\mu$g/mL) | 17 mm | 16 mm | 12 mm | 7mm | 16 mm | 12 mm | 12 mm | 7mm |

**Anti-fungal activity of aqueous extracts**

| | | *C. albicans* | | | | *A. flavus* | | | |
|---|---|---|---|---|---|---|---|---|---|
| **S. No** | **Concentration of Aqueous Extract ($\mu$g/mL)** | **(*B. variegata*)** | **(*N. nucifera*)** | **(*P. dulcis*)** | **(*T. grandis*)** | **(*B. variegata*)** | **(*N. nucifera*)** | **(*P. dulcis*)** | **(*T. grandis*)** |
| 1 | Positive control (Fluconazole 10 $\mu$g/mL) | 17mm | 14mm | 19mm | 10mm | 15mm | 17mm | 14mm | 10mm |
| 2 | 100 $\mu$g/mL | 9 mm | 7 mm | 6 mm | NIL | NIL | NIL | 6 mm | NIL |
| 3 | 200 $\mu$g/mL | 9 mm | 9 mm | 8 mm | NIL | NIL | NIL | 8 mm | NIL |
| 4 | 300 $\mu$g/mL | 10 mm | 10 mm | 10 mm | NI | NIL | NIL | 10 mm | 6mm |
| 5 | 400 $\mu$g/mL | 13 mm | 11 mm | 11 mm | 7mm | 8 mm | 7 mm | 11 mm | 7mm |
| 6 | 500 $\mu$g/mL | 16 mm | 12 mm | 12 mm | 7mm | 9 mm | 8 mm | 12 mm | 7mm |

The biologically active compounds found in *P. dulcis* (almond) extracts include polyphenols, flavonoids, and volatile compounds. Polyphenols and flavonoids have antidiabetic, antioxidantand cytotoxic effects, which make the extract suitable for medicinal use. The existence of activities like alpha-glucosidase and alpha-amylase inhibitors and anti-oxidant values assert its usefulness in combating oxidative stress, diabetes and cancer, thereby proving the pharmacological implication of these phytochemicals [18,19].

From other research highlights, lotus leaves are traditionally used for the treatment of hemorrhagic diseases including hematemesis and hematuria.The antioxidant activity of the methanol extract from the leaves of *N. nucifera* (lotus) has been studied in the work. The work also demonstrates that lotus leaf extract has effective antioxidant properties and could be used to prevent diseases resulting from antioxidant stress. These findings make a positive degree of contribution to the ongoing research to define lotus as one of the potential traditional Chinese medical plants. As the study shows, the examined leaf extracts of plants are characterized by high antioxidant activity and exhibit antimicrobial properties against a wide range of microbes. These results give evidence of lotus usage in folk medicine for its healing characteristic and opening the way for its use in new medicine [19–21].

This research clearly indicates that all the leaves have significant levels of phytoconstituents antioxidant, antimicrobial food preservation and pharmacological efficacy. Smart packaging in foods is a major concern of the latest advancements in the packaging sector, the main goal of which is to extend food shelf life with quality value. Active packaging is different from traditional packaging since they are intelligent and functions positively impact the food product and the surrounding environment. Some of the phytochemical compounds include phenols and flavonoids which have properties of natural antioxidants that can help in preventing the oxidative degradation of the food materials. Oxidation is a leading cause of quality deterioration in packaged foods through factors such as rancidity, change in colour and nutrient depletion. Likewise, the antimicrobial activities of plant derived ingredients can also be utilized in preventing the growth of food-borne pathogenic bacteria, ultimately improving the quality of the food. These dual roles make medicinal plants desirable for the synthesis of bioactive agents in green packaging utilization.

## 4 Conclusion

This study suggests the potential use of *Bauhinia variegat*a as a potential bio-food packing film. To the best of our knowledge, this study significantly differs from other similar works in the way it is interdisciplinary, which incorporates life sciences and material science to explore a crucial area: sustainable packaging. Furthermore, the study focuses on the aqueous solution which poses environmental issues and safety where the proposed applications are for the interaction with foods. This perspective is consistent with ongoing efforts to lessen the packaging sector's environmental impact and improve population health by using safer natural materials. Future work will be studying the value addition part of the plant leaves so that it can be used versatile food packing material.

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