



## *Tectona grandis* Linn. challenges and perspectives of the potential use of the species

### *Tectona grandis* Linn. Retos y perspectivas del potencial uso de la especie

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#### Abstract

*Tectona grandis* Linn f. is a species belonging to the Lamiaceae family, which grows in the Indian peninsula, Myanmar, Thailand, Lao People's Democratic Republic. The present study offers a pharmacognostic evaluation (numerical indices) and evaluates the antioxidant power of the aqueous extract of the leaves of *T. grandis*. A drying study was carried out in the sun and in an oven to determine the most suitable method, and the oven method turned out to be the most appropriate, with a maximum drying time of 30 h. The numerical indices of residual moisture, total ash, water-soluble ash and hydrochloric acid-insoluble ash were used to guarantee the quality of the plant material and establish, for the first time, these parameters for the leaves of the species. The content of total phenols was determined, with values between 75 and 310 mg GAE·gDE<sup>-1</sup>, as well as the evaluation of the reducing potential of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the antioxidant activity of the extract by the system β-carotene/linolenic acid. The extract obtained from *T. grandis* leaves showed promising antioxidant activity.

**Keywords:** *Tectona grandis*; antioxidant activity; numerical indices; pharmacognostic.

**Suggested Citation:** Lazo-Pérez, Y., Morales-Moreno, A., Rodríguez-Almeida, N. N. (2024). *Tectona grandis* Linn. challenges and perspectives of the potential use of the species. *La Técnica*, 14(1), 53-59. DOI: <https://doi.org/10.33936/latecnica.v14i1.6426>

Received: January 31st, 2024  
Accepted: February 15th, 2024  
Published: February 23rd, 2024

#### Resumen

*Tectona grandis* Linn. f es una especie perteneciente a la familia Lamiaceae, que crece en la península India, Myanmar, Tailandia, República Democrática Popular Laos. El presente estudio ofrece una evaluación farmacognóstica (índices numéricos) y evalúa el poder antioxidante del extracto acuoso de las hojas de *T. grandis*. Se realizó un estudio de secado al sol y en estufa para determinar el método más idóneo, resultando ser el de la estufa el más adecuado, con un tiempo máximo de secado de 30 h. Los índices numéricos de humedad residual, cenizas totales, cenizas solubles en agua y cenizas insolubles en ácido clorhídrico se utilizaron para garantizar la calidad del material vegetal y establecer, por primera vez, esos parámetros para las hojas de la especie. Se determinó el contenido de fenoles totales, con valores entre 75 y 310 mg EAG·gDE<sup>-1</sup>, así como la evaluación del potencial reductor del radical 2,2-difenil-1-picrilhidracilo (DPPH), y la actividad antioxidante del extracto por el sistema β-caroteno/ácido linolénico. El extracto obtenido de las hojas de *T. grandis* mostró prometedora actividad antioxidante.

**Palabras clave:** *Tectona grandis*; actividad antioxidante; índices numéricos; farmacognóstico.



## Introduction

Plants are able to produce metabolites with anti-oxidant activity, especially phenolic compounds (Haggag and Elhaw, 2022), such as flavonoids and tannins. Furthermore, natural compounds are innovative, economic, easy to access, and have reduced toxicity. The study of medicinal plants have garnered special attention for discovering new applications. The research on *Tectona grandis* Linn. (Lamiaceae) has received attention due to its diverse uses. The secondary metabolites, also known as extractives within the context of forestry products, are non-structural chemical compounds produced by plants.

Polyphenols are a group of different compounds with recognized activity, that inhibit various enzymes (Gonçalves et al., 2017; Xuan Zhou et al., 2021). Many polyphenols are also free radicals. Some authors attribute the bioactivity of the species not only to the toxicity of the extracts, but also to a synergic effect of toxic antioxidant properties (Vuolo et al., 2019). The most important secondary metabolites that have been found in the leaves of *T. grandis* (Macías et al., 2008) are apocarotenoids, abeograndinoic acid, tannins, probably of the gallotannin type, ellagitannins, derivatives of gallic, digallic and ellagic acid (Macías et al., 2010).

For some apocarotenoids, their phytotoxicity has been confirmed, as well as the allelopathic effect of lettuce and tomato (de Vargas et al., 2016). Other studies allow to isolate diterpenes and triterpenes and demonstrated the high phytotoxic activity of 2-oxokovalenic acid and 19-hydroxyferuginol (Macías et al., 2010). Some studies suggest that triterpenic acids such as oleanolic and ursolic acids or their glycosides (dichloromethane extracts) may be related to resistance to termite and fungal attacks. (Krishna et al., 2010). Lacroix et al. (2012) isolated lignans, norlignans and some phenolic compounds from dichloromethane and ethyl acetate. It is also important to note that they isolated tectonoelin A and tectonoelin B for the first time from *T. grandis*, and likewise with anthraquin under the same conditions (Lacroix et al., 2012). Nayeem y Karvekar (2010) found phenolic acids.

Because the plant material is affected by weather, soil, age and genetic conditions (Majumdar et al., 2007), it is important to obtain precise information of their chemical composition to evaluate their potential. Further, the composition of the extracts show differences from one plant to the other, and even within the same tree; therefore, the standardization of processes becomes important. The study had as the objective to evaluate the pharmacognostic parameters of the leaves from *T. grandis* and the effect of the aqueous extract on them.

## Materials and methods

### Plant material

The plant material used in the research was re-collected in the Shushufindi town, province of Orellana, between the months of September and October 2023, in the morning hours. It included the leaves of the plant from a carbonate soil (figure 1).



Figure 1. *Tectona grandis* leaf.

### Analysis of the drying process

A study of the drying process (50 g) of the leaves from *T. grandis* was carried out, which allowed to establish the most adequate method and the necessary time to conduct the process. The analysis included the traditional method of sun drying and artificial drying using an air recirculation oven (BINDER GmbH, Germany). Then, the plant material was ground with a blade grinder to obtain a particle size between 1 and 2 mm (IKA Werke MF 10, EE.UU.). The dry ground plant material was stored in polyethylene bags, inside a desiccant protected from light and humidity, until later use.

### Aqueous extract obtention

The ultrasound-assisted extraction was used, with water as a solvent (Branson, EE.UU.). In each extraction, 40 g of plant material were weighted and pulverized, then 4000 mL of distilled water were added, first wetting it with some of the solvent. The extraction lasted 1h, at a temperature of 60 °C ultra-sound power at 75%. The process was repeated until the plant material ran out, completely, and after the extraction process was finished, the resulting volume from each extraction was collected, filtered and concentrated at minimum volume using a rotary evaporator (IKA Rotary Evaporators 10 digital V., EE.UU.). Five mL were taken from the final extraction to determine the total solid content. The remaining volume was evaporated until it was dry. The performance value was

calculated with the equation, in which  $M_f$  was the final mass from the dry extract and  $M_i$  was the initial plant material used for the extraction.

$$R = \frac{M_f}{M_i} * 100 \quad (1)$$

### Residual humid content

The residual humid content was determined using the gravimetric method:

Total ash, water soluble ash and HCl insoluble ash were carried out according to USP38 (2015).

### Total phenolic content

The content of total phenolics in the aqueous extract of *T. grandis* leaves was determined using the Follin-Ciocalteu colorimetric reaction (Molole et al., 2022), with some modifications. Calibration curves (125, 250, 500 and 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ ) were prepared from the gallic acid standard up to 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  (Sigma-Aldrich). For the assay, 96-well plates were used. Once the solutions were prepared, 50  $\mu\text{L}$  of each solution was placed in the wells (in triplicate), 100  $\mu\text{L}$  of Folin-Ciocalteu reagent (1  $\text{mol}\cdot\text{L}^{-1}$ ) was added and allowed to react for 8 min. Then 80  $\mu\text{L}$  of 7.5% sodium carbonate solution was added, homogenized, and incubated at room temperature in the dark for 2 h. Absorbance was measured at 765 nm using a Variousskan™ LUX microplate reader (Thermo Fisher Scientific, Inc., Waltham.). The total content of phenolic compounds in the extract was expressed as milligrams of gallic acid equivalent per gram of extract ( $\text{mg EAG}\cdot\text{gDE}^{-1}$ ) using the equation obtained from the gallic acid calibration curve.

### Antioxidant activity of the aqueous extract

Evaluation of the reduction ability of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant activity was evaluated using the reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH), following the methodology described by (Adeku et al., 2022) with minor modifications. Methanol was used as a blank and a methanolic solution of pyrogallol (final concentration of 50  $\mu\text{g}\cdot\text{mL}^{-1}$ ) as a positive control for the assay. The aqueous extract of *T. grandis* leaves was evaluated in triplicate, with final concentrations ranging between 1 and 200  $\mu\text{g}\cdot\text{mL}^{-1}$ , in a 96-well microplate. DPPH solution (100  $\mu\text{L}$ , 300  $\mu\text{mol}\cdot\text{L}^{-1}$ ) was added to the wells corresponding to the samples, and 100  $\mu\text{L}$  of methanol was added to the wells corresponding to the control. The plate was read at  $\lambda=517$  nm at 5 minute intervals. After 30 minutes, the percentage of antiradical activity (ARA) was calculated using equation (2).

$$\%ARA = 100 - \left[ \frac{(A_{\text{in}} - A_{\text{5min}})}{A_{\text{i}}} * 100 \right] \quad (2)$$

where  $A_c$  corresponded to the absorbance of the control and  $A_m$  was the absorbance of the sample after 30 minutes. Samples with an IC50 less than 200  $\mu\text{g}\cdot\text{mL}^{-1}$  were considered active and samples with results less than 50  $\mu\text{g}\cdot\text{mL}^{-1}$  were considered promising.

### Evaluation of antioxidant activity by the $\beta$ -carotene/linoleic acid system

A  $\beta$ -carotene/linoleic acid co-oxidation system was developed for the evaluation of antioxidant activity (Chintong et al., 2019). The aqueous extract of *T. grandis* leaves was solubilized in methanol (concentration of 2.2  $\text{mg}\cdot\text{mL}^{-1}$ ) and subsequently diluted to concentrations between 125 and 200  $\mu\text{g}\cdot\text{mL}^{-1}$ . Aliquots (25  $\mu\text{L}$ ) of each concentration were added to a 96-well plate and 25 mg of linoleic acid and 100 mg of Tween 20 were transferred to a balloon containing 1 mL of  $\beta$ -carotene solution (1  $\text{mg}\cdot\text{mL}^{-1}$ ) in chloroform.

The mixture was evaporated to remove chloroform, 50 mL of aerated water was added to obtain the  $\beta$ -carotene emulsion, and 250  $\mu\text{L}$  of the emulsion was placed in the wells of the microplate. The blank was prepared according to the described methodology, but without the addition of the  $\beta$ -carotene solution. Methanol and quercetin (20  $\mu\text{g}\cdot\text{mL}^{-1}$ ) were used as negative and positive controls, respectively. The first reading was taken at 470 nm and the plate was subsequently incubated at 45 °C with readings taken every 15 minutes, for 120 minutes. The antioxidant activity was expressed as percentage of inhibition of lipid peroxidation (%I), according to equation (3) (Gouveia-Figueira et al., 2014):

$$\%I = \frac{(AbsC_{\text{initial}} - AbsC_{\text{final}}) / (AbsS_{\text{final}} - AbsS_{\text{initial}})}{(AbsC_{\text{initial}} - AbsC_{\text{final}}) * 100} \quad (3)$$

where initial AbsC was the absorbance of the control at  $t=0$  and final AbsC was the absorbance of the control at  $t=120$  min. Initial AbsS corresponded to the absorbance of the samples at  $t=0$ , and final AbsS to the absorbance of the samples at  $t=120$  minutes. Samples with results lower than 50  $\mu\text{g}\cdot\text{mL}^{-1}$  were considered promising.

### Statistical analysis

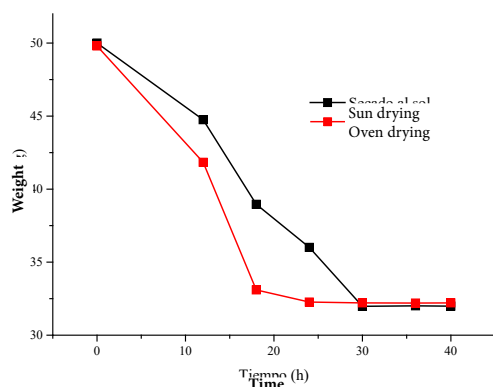
All analyzes were carried out in triplicate. Reported data were expressed as the mean of replicates. The results of the antioxidant activity assays were expressed as effective concentration to capture 50% of the radicals (IC50) determined by nonlinear regression using GraphPad Prism (GraphPad Software, San Diego, CA, USA, version 6).

### Results and discussion

The plant material was collected in areas with carbonate-rich soil located in the Shushufindi municipality of the Orellana province. This was done manually, during the early morning. The sun-drying analysis (figure 2) showed that after 30 h there was



a constant behavior in relation to biomass loss in the plant material, which indicated that the optimal drying time was 36 h under these conditions. Furthermore, an artificial drying process was done in an oven at 35 °C, and after 30 h, there was constant biomass loss in the plant material.



**Figure 2.** Analysis of drying process in the leaves of *Tectona grandis*.

Therefore, the optimal drying time for the leaves was 30h in an oven with recirculated air, because it took less time. The environmental factors did not affect the quality of the plant material because the temperature was stable and there was adequate ventilation. The process did also substantially reduced the volume and biomass of the plant material, allowing its storage for an extended period. The drying process did not significantly modify the color of the leaves, but it did affect the friability, which facilitated the subsequent particle size reduction and helped the extraction process. The dry ground plant material maintained the macromorphological characteristics of natural drugs (USP38, 15).

#### Extraction, performance calculation and residual humidity

The extraction was ultrasound-assisted, with a extractive performance of (20.95%) and high total solid content. The assay for the residual content is particularly important for plant drugs that absorb water easily, or that quickly deteriorate in its presence. When carrying out the drying process, a residual humidity content of 6.77% was obtained, which is under the limit value of (10%) (USP38, 2015). This value guaranteed an adequate quality of plant material, because it contributed to the preservation of micro-organisms and the enzyme activity that produced chemical changes that affected the quality of the material in the long term (Aware et al., 2022).

Table 1 shows the total ash content (8.99%) was high (higher than 5%), which was a representation of the inorganic composition of the leaf (Shakeel et al., 2022). The result of the ashes dissolved in the water, as it is shown in table 1, was 3.41%, which indicated the presence of metal and earth alkaline salts. The results from the insoluble ashes in HCl provided a value of 0.91%. When comparing these results with the studies carried out for other organs of the plants, the greatest, it was discovered that the greater percentage of inorganic plant material is not found in the leaves of the species.

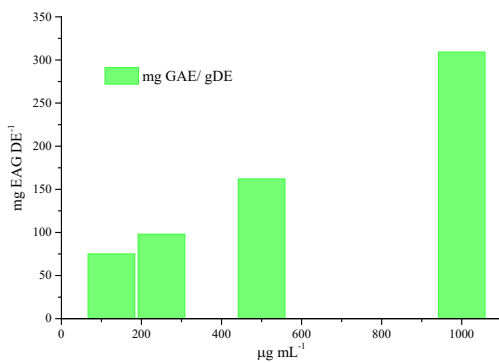
**Table 1.** Numerical indexes for the leaves of *Tectona grandis*.

Index	Results (%)
Residual humidity	6.70
Total ash	8.99
Soluble ashes in H <sub>2</sub> O	3.41
Soluble ashes in HCl	0.91

The polyphenols have an ideal chemical structure for the free-radical scavenging activity (Guo et al., 2022). The total phenolic content in the aqueous extract from the *T. grandis* leaves was calculated using the se calculó utilizando gallic acid calibration curve ( $y= 0.0099x + 0.0262$ ,  $R^2= 0.9961$ ) and was expressed as EAG·gDE<sup>-1</sup> (equivalent milligrams of gallic acid per gram of dry extract). The phenolic content of the evaluated extract ranged between 75 and 310 mg EAG·gDE<sup>-1</sup> (figura 3), which was higher than other reported values of other species in the Lamiaceae family (Bhaskara et al., 2015), which confirmed the obtained results. The main groups of polyphenolic compounds, including flavonoids and tannins, were shown in the phytochemical screening (Mama et al., 2019). The polyphenolic content reported in other parts *T. grandis* was similar to that of the aqueous extract from the leaves, which might be related to the biogenetic route used by the species for the formation of metabolites.

#### Evaluation of the reducing potential of the DPPH radical

The results from the assay are shown in table 2 and were expressed as IC<sub>50</sub>, which is the efficient concentration to decrease the initial concentration of DPPH to 50%. The aqueous extract from the *T. grandis* leaves was considered to be active, as it presented IC<sub>50</sub> values lower than 200 µg·mL<sup>-1</sup> and with an antioxidant activity when the values were lower than the established parameter of 50 µg·mL<sup>-1</sup> (IC<sub>50</sub>= 40.35 ± 0.66 µg·mL<sup>-1</sup>) (Sharma et al., 2014).



**Figure 3.** Total phenolic content in the leaves of the *Tectona grandis* species.

However, when comparing these results with the ones obtained for the pirogalol standard (IC<sub>50</sub> pirogalol, 28.60 µg·mL<sup>-1</sup>), it was observed that the values were higher for the evaluated sample. The calibration curve for pirogalol ( $y = 0.0126x - 0.11533$ ,  $R^2 = 0.9996$ ), showed a linear increase in the purifying activity when increasing the concentration given by the reaction to the electron donation between antioxidant molecules and the DPPH• radical. Considering that the low values were highly representative of an initial antioxidant activity mark (Guo et al., 2022), it can be deduced that the antioxidant activity of the analyzed extract was lower due to this antioxidant mechanism (Gomathi et al., 2011), which was seen as adequate, taking into account that the pirogalol was a pure compound, while the extract had a varied metabolite composition, but in lower concentrations

**Table 2.** DPPH• assay and evaluation by the β-carotene/linoleic acid system in the leaves of the species *Tectona grandis*.

	DPPH•µg·mL <sup>-1</sup>	βcarotene/linoleic acid(µg·mL <sup>-1</sup> )
Assay 1	40.16	18.27
Assay 2	40.63	22.05
Assay 3	39.59	16.29
Median	40.35	18.87
SD	0.66	2.93

The activity obtained for the extract by the β-carotene/linoleic acid system (table 2) showed an IC<sub>50</sub> value of  $18.27 \pm 2.93$  µg·mL<sup>-1</sup> ( $< 50$  µg·mL<sup>-1</sup>), higher than the of quercetin (IC<sub>50</sub>, 2.40 µg·mL<sup>-1</sup>). For phenolic compounds to be considered antioxidants and to exert their biological action, it is necessary that, in low concentrations, they are capable of impeding, delaying and preventing autoxidation or oxidation mediated by free radicals (Ammar et al., 2014) and that the product

formed after the reaction is stable. Previous researchers (Micera et al., 2020) isolated glycosylated flavonoids derived from quercetin, apigenin, kamferol and others from this plant, with high antioxidant activity..

### Conclusion

The analysis of the drying process of the *T. grandis* leaves showed that the best conditions for drying were at 35 °C with recirculated air, for 30 h. It was determined that the numerical indexes that allowed analyzing the quality control for the raw material that was used. The ultrasound-assisted extraction method was efficient, with a yield of 20.95%. The phenolic content of the aqueous extract from the *T. grandis* ranged between 75 y 310 mg of EAG·gDE<sup>-1</sup>. The analysis of the reduction potential of the DPPH free radical showed a IC<sub>50</sub> de  $40.35 \pm 0.66$  µg·mL<sup>-1</sup>. Meanwhile, the analysis for the antioxidant activity for the β-carotene/linoleic acid system showed a IC<sub>50</sub> of  $18.87 \pm 2.93$  µg·mL<sup>-1</sup>.

The results for the aqueous extract from the *T. grandis* leaves allowed, to some extent, to know the potential use that some species may have, and at the same time, presents new challenges for the research of this plant. It is confirmed that the analyzed extract has antioxidant activity, according to the methods that were used, obtaining comparable results with the reported values for other medicinal plants in the reviewed literature.

### Acknowledgements

The authors thank the National University of Loja (UNL) for the support granted to carry out this research..

### Conflict of interest

The authors declare that they have no conflicts of interest in this publication in any of its phases.

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### Author contribution

Authors	Contribution
Yamila Lazo-Pérez	Research desing, analysis and interpretation of data, preparing and editing the manuscript.
Arliet Morales-Moreno	Participated in the preparation of the samples.
Nancy Narcisa Rodríguez-Almeida	Lab work, literature review.

