

Measurement of Lapachol in Iranian *Tecomella undulata* (Roxb.) Seem

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ABSTRACT

Some species of *Tecoma* and *Tabebuia* (Bignoniaceae family) have been reported to contain lapachol and are used in traditional medicine. *Tecomella undulata* (Roxb.) Seem. is the only native species of the family in arid and semi-arid parts of the south of Iran. In this research, we extracted lapachol from *T. undulata* stem dried inner bark collected from near Firoozabad city in the Fars province of Iran using three extraction methods: 1) Soxhlet with direct and indirect heat for 4 hrs; 2) hot plate at 50°C for 4, 6, 8, and 10 hrs; 3) waterbath at 50°C for 25, 50, 75 and 100 hrs. The solvent in all methods was ethanol. Lapachol was detected by high performance liquid chromatography (HPLC). The most effective extraction method was the use of a waterbath for 75 hrs, yielding 3.4% (w/w) lapachol. *T. undulata* could be a candidate for traditional medicinal use.

Keywords: Bignoniaceae, HPLC, Soxhlet, stem inner bark, traditional medicine, waterbath

INTRODUCTION

Lapachol is a naphthoquinone (Pinto and de Castro 2009) isolated from *Tabebuia avellanedae* (Bignoniaceae), which is a tree growing in South America. Lapachol has also been extracted from other species of the Bignoniaceae family (de Andrade Lima *et al.* 2005; Gómez Castellanos *et al.* 2009). A wide spectrum of therapeutic activities has been attributed to lapachol or its derivatives. These include anti-abscess, anti-ulcer, antileishmanial, anticarcinomic, antiedemic, anti-inflammatory, antimalarial, antiseptic, antitumor, antiviral, bactericidal, fungicidal, insectifugal, pesticidal, protisticidal, respiradepressant, schistosomicidal, termiticidal, and viricidal (Guiraud 1994; Park *et al.* 2006; Hussain *et al.* 2007; Esteves-Souza *et al.* 2007; Lira *et al.* 2008; Salustiano *et al.* 2010). Apart from the Bignoniaceae family, lapachol can also be found in other families such as the Verbenaceae, Proteaceae, Leguminosae, Sapotaceae, Scrophulariaceae, and Malvaceae (Joshi and Singh 1977).

The Bignoniaceae is a taxon of flowering plants comprised mainly of trees, shrubs, lianas, and a few herbs. Family members are distributed mostly in the tropics and subtropics, but with a number of temperate species as well. The family includes about 650 species in 110 genera. Many of the timbers derived from members of this family contain the dermatitic compound lapachol (formerly known as tecomin). *Tecoma*, *Tabebuia* and *Tecomella* are some examples of such genera. Some species of *Tecoma* and *Tabebuia* have been reported to contain lapachol and are used in Brazil for traditional medicine (Gómez Castellanos *et al.* 2009; Pinto and de Castro 2009).

Tecomella undulata (Roxb.) Seem. (Bignoniaceae) is a deciduous or nearly evergreen large ornamental tree of arid and semi-arid regions. It occurs on flat and undulating areas including gentle hill slopes and sometimes in ravines. It is well adapted to well-drained loamy to sandy loam soil having a pH 6.5-8.0. The species thrives very well on stabilized sand dunes, which experience extremely low and

high temperatures. It grows in areas of scanty rainfall (annually 150-500 mm) and high temperature (35-48°C). It can withstand extremely low temperatures (0 to -2°C) during winter and high temperatures (48-50°C) in summer. It is drought, frost, fire and wind hardy. At the time of flowering (December-February), it produces beautiful showy yellow, orange and red flowers. Three types of flower-bearing trees can be observed close to each other in the same vicinity. Distribution of *T. undulata* is restricted to the drier parts of Arabia, Pakistan, Afghanistan, northwest India and the south of Iran up to an elevation of 1200 m. *T. undulata* is mainly used as a source of timber. Its wood is strong, tough and durable. *T. undulata* plays an important role in environmental conservation, for example, in the protection of soil from flood water, and enhances the productivity of associated plants by improving soil water in the upper soil layer, soil organic matter and N availability (Singh 2009; Mohsenzadeh *et al.* 2010; Sayyadnia Tayyebi 2010).

Lapachol can be extracted by methanol or ethanol and then determined and separated by high performance liquid chromatography (HPLC) (Steinert *et al.* 1995). The objective of this study was to extract lapachol from the dried stem inner bark of *T. undulata*, distributed in the south of Iran, using three different methods.

MATERIALS AND METHODS

Plant material

The bark of *T. undulata* stems was harvested from five trees approximately 20-years old distributed around Firoozabad city in the Fars province of Iran. A voucher specimen (Mohsenzadeh 1002) was deposited in the herbarium of Shiraz University. The inner bark was separated and dried at 70°C for 24 hrs using an oven and then was ground to a fine powder before extraction. Lapachol was extracted using 2.5 g of powder in 50 ml of solvent by three methods: 1) Soxhlet with direct and indirect heat for 4 hrs; 2) hot plate at 50°C for 4, 6, 8, and 10 hrs; 3) water bath at 50°C for 25,

50, 75 and 100 hrs. The extracts were initially passed through a simple paper filter followed by a 0.45 µm membrane filter. Then, 0.5 ml of extract was diluted with ethanol to 5 ml. Typically, 5 µl of filtrated and diluted extracts were injected into HPLC for the direct separation and quantification of lapachol.

HPLC determination

Pure lapachol as standard material was purchased from Aldrich (Germany). It was dissolved in methanol to give a concentration of 1000 mg/ml and then samples with a concentration of 30 to 100 mg/ml were injected into the HPLC. The methanol was HPLC grade (Merck, Germany) and ultra pure water was obtained from a Milli-Q water system. Quantification was performed through comparison of the respective peak areas resulting from the extracts and reference solutions. The elution time of the standard using the HPLC was 3.46 min.

The chromatographic equipment consisted of two PU-2080 plus delivery pumps (Jasco, Japan), an injector valve Rheodyne 7725i (USA) with a 5-µl loop sample, and a variable wavelength absorbance detector UV-2070 plus (Jasco) operating at 340 nm. The chromatographic system was controlled by an HSS-2000 provided by Jasco using an LC-Net II/ADC interface. Data was processed using BORWIN software (version 1.50). Isocratic separations were carried out at room temperature on a Finepak SIL C18, 100 Å pore size, 10 µm particle size, 250 × 4.6 mm ID. A mixture of acetonitrile: water (50: 50 w/w) at a flow rate of 1.0 ml/min was used as the mobile phase. A Sartorius mechanical balance with 10 µg interpolating resolution was used for weighing the samples (Viana *et al.* 2003).

Statistical analysis and computations

All experiments were set up in a randomized complete block design with three replicates each. Raw data was imported to Windows Microsoft Excel for calculations and graphical representation. Anova 1 from SPSS software (version 17.0) for analysis of variance and Duncan's multiple range test for significant differences between means were used. Powder weight of used stem inner bark, dilution rate, peak area of HPLC graphs and standard curve were used to calculate the percentage of lapachol (w/w) in the dry weight of stem inner bark.

RESULTS AND DISCUSSION

In **Fig. 1**, peak areas of different concentrations of pure lapachol are plotted against the concentration of acetonitrile in the mobile phase. A significant linear correlation between peak area and concentration of lapachol is evident.

In addition, a chromatogram of pure lapachol injected into the C18 column is illustrated in **Fig. 2**. Lapachol concentrations of extracted solutions from stem inner bark of *T. undulata*, with Soxhlet, hot plate method and waterbath methods at 50°C for 25, 50, 75 and 100 hrs are shown in **Table 1**. The low lapachol content when the Soxhlet with directed heat and hot plate methods were used may be related to decomposition of lapachol by direct heat (Babula *et al.* 2009). In addition, the decrease in lapachol content following waterbath extraction at 50°C for 100 hrs compared to 75 hrs indicates that prolonged heat had a negative effect on lapachol. When a Finepak SIL C18 end capped stationary phase with a very low metal content was selected for analyzing lapachol, the lapachol peak was symmetrical (As = 1.2). This showed that the conditions for lapachol separation by column were good.

As shown in **Fig. 2**, lapachol was eluted 3.46 min after injection. As expected, an increase in the molar fraction of acetonitrile of the mobile phase resulted in decrease in the retention time of lapachol and thus shortened the analysis period. All the experiments were carried out at 50% acetonitrile in the mobile phase as this was previously found to be the most suitable composition (Mohsenzadeh *et al.* 2010). At higher percentages of acetonitrile, the lapachol peak overlaps with the peak of other components. A comparison of the three extraction methods by peak area and lapachol

Table 1 Lapachol amounts of *T. undulata* stem inner bark and variance analysis of extraction methods.

Extraction method	Lapachol % (w/w)
Soxhlet with undirected heat	0.50 ^{d*}
Soxhlet with directed heat	0.01 ^e
Hot plate at 50°C	0.01 ^e
Waterbath at 50°C for 25 hr	0.47 ^d
Waterbath at 50°C for 50 hr	1.50 ^b
Waterbath at 50°C for 75 hr	3.37 ^a
Waterbath at 50°C for 100 hr	1.30 ^c

* Different letters show significant differences at $P < 0.05$ according to DMRT.

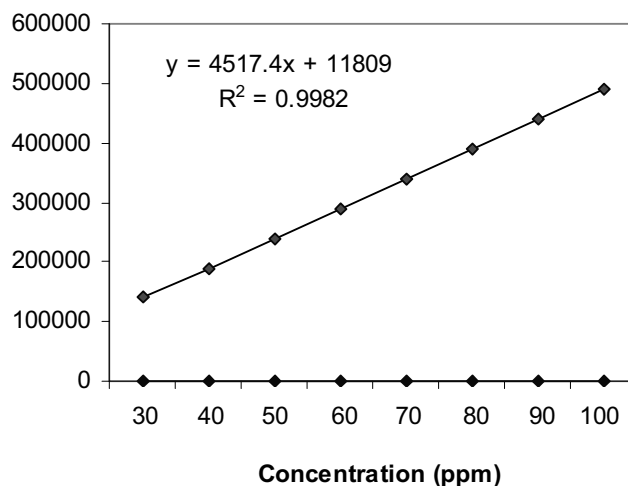


Fig. 1 Calibration curve.

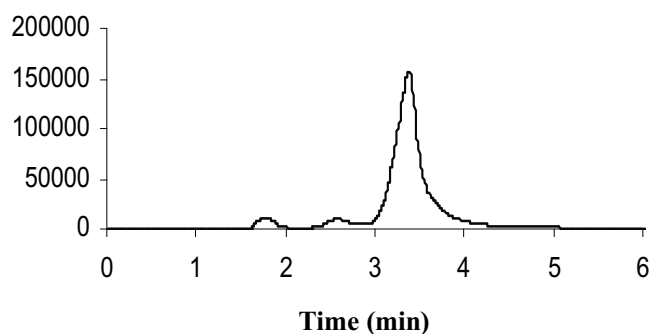


Fig. 2 Chromatogram of pure lapachol.

concentration demonstrated that the most effective extraction method for isolation of lapachol was the waterbath method at 50°C for 75 hrs. Extraction using a waterbath is an economical and effective extraction method. According to this method, inner bark of *T. undulata* had 3.4% lapachol based on dry weight but only 0.5% when the Soxhlet method was used. *T. avellanadae*, commonly termed Paud'arco in Brazil, and which has traditional medicinal uses in China (Pereira *et al.* 2006) had 2% lapachol based on dry weight of wood and 7% lapachol from stem inner bark (Viana *et al.* 2003), both using the Soxhlet method. In a previous study, we extracted lapachol from *T. undulata* by three methods: heating methanol in a microwave for 6 min, non-heated methanol and hot water (90°C). The most effective extraction method was the first method with 3.6% lapachol dry weight from the stem inner bark (Mohsenzadeh *et al.* 2010). Variation of lapachol levels may be related to postharvest alteration of naphthoquinone content as mentioned by Awang *et al.* (1995).

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