



## Cytotoxic Effects of *Tabebuia Rosea* Oils (Leaf and Stem Bark)

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### Abstract

The leaf and stem bark of *Tabebuia Rosea* were separately collected, dried and grounded. The powdered samples were subjected to distillation using a hydro-distiller (all-glass clevenger apparatus), to extract the essential oil present in the plant samples. GC and GC/MS analysis were carried out on the essential oils and were found to contain a total of five and six compounds in the leaf and stem bark respectively. The leaf contained *o*-xylene (2.13%), 2,4-dimethylhexane (1.03%), methyl cyclohexane (53.13%), methyl benzene (12.75%), 3-Pentene-2-one (0.11%) representing 69.15% of the total essential oil while the stem bark contained *n*-amyl ketone (46.69%), methyl cyclohexane (24.07%), methyl benzene (13.88%),  $\alpha$ -carene (0.46%),  $\beta$ -carene (0.46%), and  $\gamma$ -carene (0.46%) representing 85.62% of the total essential oil. The toxicity of these oils was shown by brine shrimp test. The  $LC_{50}$  value ( $\mu\text{g/ml}$ ) of 1.701 with upper confidence limit and lower confidence limit of 2.137 and 0.4678 respectively for both the leaf and stem bark indicated that the oils were highly toxic.

**Key words:** *Tabebuia rosea*, cytotoxicity, hydrodistillation, Gas Chromatography/ Mass Spectroscopy.

### INTRODUCTION

*Tabebuia rosea* (Bignoniaceae) is a huge canopy tree native to the Amazon rainforest and other tropical parts of South and Latin America. It is a deciduous, massive and majestic tree. It is commonly known as "Pink Trumpet tree" and can grow up to 15 meters high. It is well known for its beautiful flowers and can live for hundreds of years. The fruits are green, long and bean pod-like with a length of 20-40cm. The fruits turn dark brown when ripe and contain flat, heart-shaped seeds with tiny wings. It has other common names like *pau d'arco*, *ipê roxo* and *lapacho*. It became popular in Nigeria due to its various medicinal applications traditionally; as astringent, anti-inflammatory, antibacterial, antifungal, and laxative; it is used to treat ulcers, syphilis, urinary tract infections, gastrointestinal problems, candida and yeast infections, cancer, diabetes, prostatitis, constipation, and allergies. Tea made from the leaf and bark has fever-reducing effect [1-2]. *Pau d'arco* and its chemicals have also demonstrated *in vitro* antimicrobial and antiviral

properties. The bark extracts of pau d'arco have also been shown to demonstrate anti-inflammatory activity [3-5]. The plant contains a large amount of chemicals known as quinoids, and a small quantity of benzenoids and flavonoids [6-7]. Several different species of *Tabebuia* trees are used interchangeably in herbal medicine. *T. impetiginosa* is known for its attractive purple flowers and is often called "purple lapacho". This paper however reports the cytotoxic activity of the essential oils of the leaf and stem bark of *Tabebuia Rosea*.

## MATERIALS AND M ETHODS

### Materials

#### Plant Materials

The leaf and stem bark of *Tabebuia rosea* were collected at the Botanical Gardens, University of Ibadan. Specimens were identified at the Forestry department, University of Ibadan, Oyo State, Nigeria. The plant materials were air dried in a shady and aerated room until the weight was stable and ground into fine powder and kept in a non-absorptive sack for subsequent use.

### Method

#### Isolation of Essential Oils

The oils were obtained by hydrodistillation on a Clevenger type apparatus for 3 h in accordance with the British Pharmacopeia specifications (1980). The essential oils were collected, dried over anhydrous sodium sulphate and stored at 4° C until analysis. The oil yield was calculated relative to the dry matter.

#### Analysis of the Essential Oils

##### *Gas chromatography*

The oils were analyzed by GC using a Shimadzu model QP2010 chromatograph. An HP-Innowax FSC column (30 m x 0.25 mm, with 0.25 µm film thickness) was used with Helium as carrier gas at a flow rate of 1 ml/min. The GC oven temperature was kept at 60°C (hold for 0 min), and programmed to reach 140°C at a rate of 5°C/min, then kept constant at 280°C for 10 min being the final hold time. The split ratio was adjusted to 50:1. The injector temperature was set at 200°C. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250°C. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentages of the characterized components are given in Table 1.

##### *Gas chromatography–mass spectrometry*

The essential oils were analysed by GC-MS using a Shimadzu model QP2010 gas chromatograph system with split/splitless injector interfaced to a 5973 mass selective detector. Innwax FSC column (30 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (1 ml/min). GC oven temperature and conditions were as described above. The injector temperature was at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 30 to 500. Library search was carried out using the commercial resources Wiley GC/MS Library, Mass Finder and the in-house Baser Library of Essential Oil Constituents.

##### *Identification of Components*

Identification of constituent of the oil was achieved on the basis of their retention indices determined with a reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns (NIST database/chemstation data system) with data previously reported in literature [8-10].

**Brine shrimp lethality test**

The brine shrimp lethality test (BST) was used to predict the presence, in the oils, of cytotoxic activity [11]. The shrimp's eggs were hatched in sea water for 48h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (1000, 100, and 10 $\mu$ g/ml) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24h the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration killing fifty percent of the larvae (LC<sub>50</sub>) was determined using the Finney computer programme.

**RESULTS AND DISCUSSION**

The oils obtained from *Tabebuia rosea* (leaf and stem bark) are light yellow oil with pungent smell. The yield of the volatile oils obtained from the leaves and stem bark of *Tabebuia rosea* were relatively low 0.24% for the leaf and 0.072% for the stem bark. This could be attributed to a lot of factors such as age of the plant, period of collection, drying and distillation temperature. A total of five and six compounds were detected and identified from the oil fraction of *Tabebuia rosea* leaf and stem bark respectively by spectral comparison (Table 1).

**Table 1: Composition of the volatile oil from the leaf and stem bark of *Tabebuia rosea* by GC-MS analysis\***

Peak no	Compound	RRI	% composition Leaf	% composition Stem bark
1	3-Pentene-2-one	622	0.11	-
2	2,4-dimethylhexane	688	1.03	-
3	n-amyl ketone	705	-	46.29
4	Methyl cyclohexane	781	53.13	24.07
5	Methyl benzene	794	12.75	13.88
6	$\alpha$ -careen	1015	-	0.46
7.	$\beta$ -carene	1015	-	0.46
8	$\gamma$ -carene	1015	-	0.46
9.	o-xylene	907	2.13	-
	Total		69.15%	85.62%

\*Percentages calculated from flame ionization detection data. RRI, relative retention indices calculated against *n*-alkanes

The leaf contains o-xylene (2.13%), 2,4-dimethylhexane (1.03%), methyl cyclohexane (53.13%), methyl benzene (12.75%), 3-Pentene-2-one(0.11%) while the stem bark contains n-amyl ketone (46.69%), methyl cyclohexane (24.07%), methyl benzene (13.88%),  $\alpha$ -carene (0.46%),  $\beta$ -carene (0.46%), and  $\gamma$ -carene (0.46%) (Table 1). The toxicity of these oils was shown by brine shrimp test. The LC<sub>50</sub> value ( $\mu$ g/ml) of 1.701 with upper confidence limit and lower

confidence limit 2.137 and 0.4678 respectively for both the leaf and stem bark indicate that the oils are highly toxic. To a very large extent, the phonological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction are possible sources of variation for the chemical composition and toxicity of the oils.

### CONCLUSION

A total of five and six chemical components were detected by GC and GC/MS in *Tabebuia rosea* (leaf and stem bark) oil respectively and were identified by spectral comparison to be mainly hydrocarbons. Brine shrimp lethality test was carried out to know the toxicity of the oils to living organisms (shrimps). The oil of *T. rosea* was discovered to be toxic. The toxicity was assayed using brine shrimps at 10, 100, and 1000ppm and LC<sub>50</sub> value (µg/ml) of 1.701 was obtained. It can therefore be suggested that its long term use may cause serious side effects. *T. rosea* used in this study was chosen on the basis that it is used traditionally for treatment of a wide array of disease conditions. The study is premised on justifying its use in traditional medicine. This work, however, shows that further investigations on the essential oil and the evaluation of the biological activities of *Tabebuia* species growing in Nigeria should be initiated. Also further studies should be done to determine the real potential for their clinical application.

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