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Chemical composition and cytotoxic effect of *Largerstroemia speciosa* fruits essential oils

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ABSTRACT

The fruits of *Largerstroemia speciosa* were collected, dried and grounded. Essential oils of powdered samples were obtained by hydrodistillation and analyzed by GC and GC/MS. The essential oils contained mostly hydrocarbons: Methyl cyclohexane (60.9%), methyl benzene (18.2%), o-xylene (3.04%) representing 82.14% of the total essential oil. The cytotoxicity result of LC_{50} value (µg/ml) of 1.701 obtained through the brine shrimp toxicity assay indicated that the oil is toxic.

Keywords: *Lagerstroemia speciosa*, cytotoxicity, hydrodistillation, Gas Chromatography/ Mass Spectroscopy.

INTRODUCTION

Herbal medicine has been employed by many people and culture throughout the world to treat diseases throughout the history of mankind. This situation still persists in many developing countries where two-third of the people have no access to modern medicine. In the industrialized countries also, there is resurgence in the use of herbal drugs and in fact, 25% of all prescribed drugs are substances derived from higher plants (Okigbo et al., 2000). Plants are without doubt the most significant and accessible sources of natural products which are biologically and medicinally important. Since prehistoric time man has used crude plant extracts to heal and to kill. Folklore abounds in references to the use of plant extracts in the healing of a variety of illness. In recent years, a great deal of attention is being paid to plant extracts for the isolation of various bioactive compounds based on their ethno pharmacological claims, that is why traditional medicine is now recognized by the World Health Organization (WHO) as a building block for Primary Health Care (Prajapati et al., 2003). Scientific interest in medicinal plant is therefore on the increase in recent times because of increased efficiency of new plants derived drugs and rising concerns about the side effects of modern medicine (Christophersen, 1991).

Banaba is the common name of the herb *Lagerstroemia speciosa*, which belong to the family *Lythraceae*, and order of *Myrtale*. It is a large tree that has a large ornamental leaves which are oblong and pointed at the ends. It grows as tall as 20 meters in height. The flowers are scentless, bright pink to purple, and the stamens wrinkled. The fruits are woody and contain seeds that are mainly winged. *Banaba* leaf has traditionally been used as remedy for the treatment of diseases

associated with elevated blood glucose levels (Burkill, 2000). The dried and shredded *banaba* leaves are known to be used as a treatment for diabetes and kidney disease (Liu et al., 2001). Scientists have identified different components of *banaba* to be responsible for its anti-diabetic activity. Using tumor cells as a cell model, corosolic acid was isolated from the methanol extract of *banaba* and shown to be an active compound. Chemical compounds identified by the bioassay-directed isolation from the leaves *L. speciosa* include ellagitannins, ellagic acid and its derivatives (Liu et al., 2001). The aim of this study was to investigate the cytotoxic activity of the essential oils of the fruits of *L. speciosa growing in Nigeria* and to identify the chemical constituents.

MATERIALS AND METHODS

Plant materials

Fruits of *L. speciosa* were collected at the Botanical Gardens, University of Ibadan. Specimens were identified at the Forestry Research Institute Ibadan, Oyo State, Nigeria (FHI No 12761). The plant material was air dried in a shady and aerated room at 25 °C until the weight was stable and ground into fine powder and kept in a non-absorptive sack for subsequent use.

Isolation of essential oils

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

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 ^{o}C (hold for 0 min), and programmed to reach 140 ^{o}C at a rate of 5 ^{o}C/min, then kept constant at 280 ^{o}C for 10 min being the final hold time. The split ratio was adjusted to 50:1. The injector temperature was set at 200 ^{o}C. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250 ^{o}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. Innowax 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 ^{o}C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 30 to 500.

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 (Adams, 2001; Joulain and Konig, 1998; Mclafferty and Staufer, 1989).

Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence, in the oils, of cytotoxic activity (Meyer et al., 1982). The shrimp's eggs were hatched in sea water for 48 h at room temperature. The *nauplii* (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were diluted in DMSO, at varying concentrations (1000, 100, and 10 µg/ml) and incubated in triplicate vials with the brine

Table 1: Chemical composition of the volatile oil from the fruits of *Lagerstroemia speciosa* by GC and GC/ MS analysis*.

Peak no	Identifeid Compounds	RRI	Peak Area (%)
1	Methyl cyclohexane	781	60.9
2	Methyl benzene	794	18.2
3	o-xylene	907	3.1
	Total		82.14

*Percentages calculated from flame ionization detection data. RRI, relative retention indices calculated against *n*-alkanes
*GC/MS-Gas Chromatography/Mass Spectroscopy.

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 24 h, 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_{50}) was determined using the Finney computer programme.

RESULTS AND DISCUSSION

The air dried crushed fruits of *L. speciosa* were utilized to obtain volatiles by means of hydrodistillation. The essential oils, light green oil with pungent smell were further analyzed both by GC and GC/MS systems using a polar column, resulting in the identification of only 3 constituents in the hydrodistilled sample, representing 82.14% of the total essential oil. The essential oil yield was 0.068% (w/w) which is low. The oil yield of *L. speciosa* seems to depend on the nature of parts of plant used for extraction and also on the mode of extraction. Overall, hydrocarbons were found in the sample as the dominating group of compounds. Methyl cyclohexane (60.9%), methyl benzene (18.2%) and o-xylene (3.04%) (Table 1) were identified as the main constituents for the hydrodistilled samples. Brine shrimp have been used as a "benchtop bioassay" for the discovery and purification of bioactive natural products and they are an excellent choice for elementary toxicity investigations of consumer products (Lieberman, 1999). Brine shrimp, *Artemia* species, also known as sea monkeys, are marine invertebrates about 1 mm in size. Cytotoxicity study on the oil was therefore done using *Artemia salina* (Brine shrimp eggs). At the various concentrations used (1000, 100, and 10 µg/ml), it was observed that the oil was toxic. The LC_{50} (µg/ml) results of 1.701 from the Brine shrimp toxicity assay with upper confidence limit and lower confidence limit of 2.137 and 0.4678 respectively further corroborated the presence in the oil hydrocarbon molecules thereby accounting for the high toxicity of the oil. This result however is in agreement with toxicity expected of biologically active chemical substance (Oloyede et al., 2010; Onocha et al., 2003).

Conclusion

A total of three chemical components were detected by GC and GC/MS in *L. speciosa* oil 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 oils of *L. speciosa* were found to be toxic. The toxicity was assayed using brine shrimps at 10, 100, and 1000 ppm with LC_{50} value (µg/ml) of 1.701. *L. speciosa* used in this study was chosen on the basis that it is used traditionally for treatment of conditions such as diabetes and kidney diseases. 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 *Lagerstroemia* species growing in Nigeria should be initiated. Also, further studies should be done to determine the real potential

for their clinical application. As a renewable bio resource, *Lagerstroemia* species can serve as a good source for natural medicines with a traditional background.

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