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Chemical Composition and Insecticidal Activity of Volatile Oil of *Khaya grandifoliola*

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ABSTRACT

Khaya grandifoliola has been used in traditional medicine against infections, rheumatic and parasitic diseases. The essential oil (EO) from the stem bark of *K. grandifoliola* was extracted with *n*-hexane, analysed by GC and GC-MS. The insecticidal activity of the EO was evaluated against two insects, *Rhyzopertha dominica* and *Tribolium castaneum* using an established standard procedure. The chemical composition of the EO obtained through GC-MS revealed 24 components. The major components were α-pinene (10.56%), limonene (1.25%), β-carophyllene (3.87%), β-pinene (7.80%), α-phellandrene (7.45%), and citronellol (5.10%). The EO exhibited significant insecticidal activity against *Rhyzopertha dominica* and *Tribolium castaneum* (85 and 80%, respectively).

Keywords: essential oil, plant extract, GC-MS, *Rhyzopertha dominica*, *Tribolium castaneum*

INTRODUCTION

The use of plant products in the treatment of diseases and infections is as old as man. About 80% of the world population relies on plants and plant extracts for their healthcare needs. A recent report indicates that of the top 150 drugs used in the USA 57% of them contain at least one major active compound derived from natural products (Rosenthal 1997; Joseph *et al.* 2008). Not only do higher plants continue to serve as important sources of new drugs, but phytochemicals derived from them are also extremely useful as lead structures for synthetic modification and optimization of bioactivities.

Tropical rainforests provide many medicinal plants with potential medicinal properties but the ethno and phytopharmaceuticals of these medicinal plants have not been fully exploited (Baladrin *et al.* 1993; Meena 2009). The potential medicinal importance is due not only to the species richness of the tropical flora, but also to the diversity of pathogens, parasites and herbivores against which the plants must provide a defensive mechanism. Many of the defense chemicals secreted have been found to be useful in the treatment and prevention of diseases affecting humans and other organisms as a result of their metabolic precision (Farnsworth 1988). Essential oils (EOs) are made up of different volatile components and have been shown to possess alot of biological activities (Ahmet *et al*. 2005).

Khaya grandifoliola, family Meliaceae, commonly called African mahogany (Hutchinson and Dalziel 1978), is widely distributed across West Africa from the Guinea coast to Cameroon and extending eastward through Congo Basin to Uganda and some parts of Sudan. It grows up to 40 m high and 5 m in girth. The bark is grey in colour and exudes a bitter gum when wounded. The aqueous decoction of the plant is bitter and used to treat many diseases.

Khaya spp. are a valuable indigenous traditional medicine in West Africa. Its bitter bark is used for various ethnomedicinal purposes such as fever, lumbago, cough, rheumatism, stomach ache, gastric pains and diarrhoea in horses and camels (Dalziel 1994). Its antimalarial, antiulcer, antianaemic and hypoglyceamic and hypocholesterolaemic properties have been reported (Agbedahuns *et al*. 1998; Bumah 1998; Bickii *et al*. 2000; Makinde *et al*. 2006; Njifutie and Njikam 2006). Some of the chemical constituents reported include limonoids (Zhan *et al.* 2008). Recently, the effect of stem bark of the plant was evaluated against methicillin-resistant *Staphylococcus aereus* and was observed to be very effective against different strains of the organisms at the tested doses (Uzoekwe *et al.* 2008).

Higher plants are a rich source of novel natural compounds that can be of great usefulness in developing environmental safe methods for insect control (Amason *et al*. 1989). Insecticidal activity of many plants against several insect pests has been demonstrated (Isman 2000; Carlini 2002).

Tribolium castaneum (Herbst) is considered as a major pest of stored grains (Howe 1965). Annual post harvest losses resulting from insect damages, microbial deterioration are estimated to be 10-25% of worldwide production (Adams 2001). The lesser grain borer is characterized as both an internal and external feeder, considered to be a serious pest of both whole kernel stored grain and cereal products. The adults and larvae bore into undamaged kernels of grain, reducing them to hollow husks. They are also able to survive and develop in the accumulated "flour" produced as the seeds are chewed up. The lesser grain borer is primarily a pest in stored wheat and corn, but it can infest tobacco, nuts, beans, bird seed, biscuits, cassava, cocoa beans, dried fruit, peanuts, spices, rodenticide baits, and dried meat and fish (Koehler and Pereira 1994).

With a rising concern for environmental safety there has been renewed interest in the use of naturally occurring insecticides including medicinal plant products (Isman 2006).

Therefore the aim of this study was to determine the chemical composition of the EO isolated from the stem of *K. grandifoliola* by GC-MS analysis and to evaluate the insecticidal activity of its EO against known insects causing severe damage to plants. To the best of our knowledge the separation and identification of the volatile components of this plant using GC-MS and chemical methods have not been previously examined.

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MATERIALS AND METHODS

Collection and identification of plant material

Fresh stem bark of *K. grandifoliola* was collected from a local government area in Benue State, Nigeria around April, 2008. The plant was identified by Mr. Sunny A of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Extraction and isolation of EO

The plant was cut into smaller pieces, dried and reduced to a fine powder with the aid of a mechanical grinder. The powdered sample (200 g) was extracted with *n*-hexane by maceration for 48 h at room temperature. The extract was evaporated to dryness using a rotary evaporator at reduced pressure. The EO was passed over anhydrous Na_2SO_4 to remove moisture. The fraction obtained was stored in a refrigerator at -4°C in the dark.

Identification of chemical components

Identification of the chemical constituents was done by a computer library MS searches using retention indices as a pre-selection routine and visual inspection of mass spectra from the literature for confirmation (British Pharmacopoeia 1980; Julian and Konig 1988; Adams 1989, 2001).

Gas chromatography

Quantitative and qualitative data were determined by GC and GC-MS respectively. The EO was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/splitless injector. The column used was a DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 µm df, coated with 5% diphenyl - 95% polydimethylsiloxane, operated with the following oven temperature programme: 50°C, held for 1 min, rising at 3°C/min to 250°C, held for 5 min, rising at 2°C/min to 280°C, held for 3 min; injection temperature and volume, 250°C and 1.0 µl, respectively; injection mode, split; split ratio, 30: 1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280°C; hydrogen, flow rate, 50 ml/min; air flow rate, 400 ml/min; make-up (H_2/air), flow rate, 50 ml/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu).

Gas chromatography-mass spectrometry

Agilent 6890N GC was interfaced with a VG Analytical 70-250s double - focusing Mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: Ionization voltage 70eV, ion source 250°C. The GC was fitted with a 30 m × 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of the GC analysis.

Insects

Insects (*Tribolium castaneum*, *Rhyzopertha dominica*) used for the test were reared in glass jars at 27 ± 1°C and 60 ± 5% relative humidity (RH) in the laboratory of the H.E.J Research Institute of Chemistry in Karachi, Pakistan.

Insecticidal activity

To test insectividal activit, test insects' volatile organic solvent (ethanol), standard insecticide (Permethrin), Petri dishes (9 cm diameter), micropipette (1000 µl), growth chamber, test sample, filter paper, glass vials and a brush are required.

Test: The EO of *K. grandifoliola* was screened for insecticidal property against *R. dominica* and *T. castaneum* in comparison with commercial insecticide Permethrin and ethanol (negative control). Insecticidal activity was determined using established standard experimental procedures (Atta-ur-Rahman *et al*. 1997).

In evaluating the insecticidal activity of the EO, a preliminary test was carried out in which several doses were chosen among those having no killing effect on the experiment population to a minimal of 100% death of this population. Using a micropipette (Rainin magnetic assist), a precise volume of EO was added to ethanol (negative control) and diluted to 5 ml. From this 0.5 ml of solution was uniformly applied to a 9 cm disk of filter paper (Whatman No.1) and placed in a Petri dish. Twenty adult insects, less than 1 month old, were introduced into the dish 5 min later and the dish was covered. A control with ethanol alone was used. For each preparation five replications were made. The number of dead insects was determined 24 h after application.

The percentage mortality was calculated by:

$$\text{Mortality (\%)} = \left(\frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \right) \times 100$$

Data analysis

Statistical analyses were performed using SAS statistical package 1994. Users guide, Version 8.2. SAS Institute Inc., Cary, NC, USA. Data are expressed as means ± standard error (SEM). Comparison was done using one-way Analysis of Variance. The value of 0.05 level of probability was chosen as a criterion for significance.

RESULTS AND DISCUSSION

The EO obtained from the stem bark of *K. grandifoliola* has a light yellow colour and fragrant aroma. The EO yield is 1.56% of dry plant material. The chemical components presented in **Table 1** were identified unambiguously by direct comparison (mass fragmentation and retention index) with reported data as well as a computer library search. The components identified were mainly oxygenated monoterpenes and sesquiterpenes hydrocarbons. The sequiterpene hydrocarbon accounted for 51.35% of the total while the oxygenated components were 19.47%. The remaining compounds were fatty acids. As shown in **Table 1**, 24 compounds were identified in the EO.

The EO also showed significant insecticidal activity (**Table 2**) against *Rhyzopertha dominica* and *Tribolium castaneum* with 80 and 85% activity, respectively.

The insecticidal activity observed is likely due to the relatively high concentrations of limonene in the EO and also partly due to the presence of α-pinene, β-pinene and β-caryophyllene in the EO. The synergistic activity of the div-

Table 1 Chemical composition (%) of EO of *K. grandifoliola*.

No	RI*	Compounds	Relative %
1	923	Thujene	2.56
2	945	Camphene	2.67
3	970	*α*- Pinene	10.56
4	977	*β*- Pinene	7.80
5	984	*β*-Myrecene	3.26
6	995	α-Phellandrene	7.45
7	1005	Δ^3-Carene	3.45
8	1012	*p*-cymene	2.42
9	1019	Limonene	1.25
10	1049	Terpene	3.57
11	1464	*β*- Carophyllene	3.87
12	1467	*Carophyllene oxide*	1.34
13	1130	Camphor	trace
14	1161	*β*-Terpinol-4-ol	0.80
15	1172	α- Terpineol	4.56
16	1212	Citronellol	5.10
17	1260	α- Terpenylacetate	1.35
18	1377	Isoledene	0.84
19	1389	*β* –cubebene	trace
20	1477	Naphthalene	4.52
21	1565	*β*-Carophyllene oxide	5.54
22	1787	Tetradecanoic acid	2.39
23	1821	1,8-cineole	0.78
24	1963	Hexadecanoic acid	12.32
Total			88.40

*Retention indices of DB-5 fused column

Table 2 Insecticidal activity of volatile oil of *K grandifoliola*.

Treatments	Conc. (μg/cm^3)	Effect of sample (%)
EO of kg [a]	1019.1	85 ± 1.34 (T.C [b])
EO of kg [a]	1019.1	80 ± 1.00 (R D [c])
Permethrin (positive control)	237.5	100 ± 0.00
Ethanol (negative control)		-

[a] *Khaya grandifoliola*
[b] *Tribolium castaneum*
[c] *Rhyzopertha dominica*

ersity of major and minor chemical constituents present in the EO is important in considering the desired biological activity.

The insecticidal activity of the EO is dependent on the active chemical constituents and the gross sensitivity of the target insects to the active chemical principle (Obeng *et al.* 1997; Rachid *et al.* 2006). The EO of *K. grandifoliola* contains limonene. Limonene is an active insecticidal chemical constituent, which has been reported to have toxic effect on Coleopterans (Tanpojou *et al.* 2002). Enatiomers of α-pinene and β-pinene and limonene have been reported to have potent antibacterial and insecticidal activity (Megiatis 1999; Tzakou *et al.* 2001; Filopiwicz 2003). α- and β-Pinene are able to destroy cellular integrity, and thereby inhibit respiration and ion transport processes. The antimicrobial activity of caryophyllene and caryophyllene oxide has been demonstrated (Ulubelen 1994; Azaz 2002; Deba *et al.* 2008) although insecticidal action is yet to be determined.

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