



## Chemical compositions and mosquito repellency of essential oils from *Artabotrys hexapetalus* and *Artabotrys rupestris*

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### ABSTRACT

The two Tanzanian *Artabotrys* species were investigated to establish the chemical composition and mosquito repellent properties of their essential oils. Hydrodistillation of leaves and stem bark of *Artabotrys hexapetalus* (L.f.) (Annonaceae) and stem bark of *Artabotrys rupestris* Diels (Annonaceae) gave essential oils which were analyzed by Gas Chromatography – Mass Spectrometry (GC-MS). While the oil from the stem bark of *A. hexapetalus* contained high amount of  $\beta$ -caryophyllene oxide, the oil from the leaves had both  $\beta$ -caryophyllene oxide and 11-hexadecyn-1-ol as the main components. The oil from the root barks of *A. rupestris* contained large amount of 6-(*p*-tolyl)-2-methyl-2-heptanol and (-)-spathulenol.  $\beta$ -Caryophyllene oxide, cadinol, cubenol, (-)-spathulenol and 7-methyl-4-methylene-1-(1-methylethyl)-naphthalene were identified as common constituents of the essential oils from the two plant species investigated. On evaluation for mosquito repellency, the essential oil from the leaves of *A. hexapetalus* was found to exhibit strong activity with  $RC_{50} = 1.81 \times 10^{-5}$  mg/cm<sup>2</sup> and  $2.79 \times 10^{-5}$  mg/cm<sup>2</sup> for the first and the second fractions, respectively against female *Anopheles gambiae* s.s. mosquitoes. The repellency activity of essential oil from leaves was significantly higher than from stem bark, but less than that of the standard citronella oil used ( $RC_{50} = 4.1 \times 10^{-6}$  mg/cm<sup>2</sup>). The observed high mosquito repellent activity for the essential oil fractions from *A. hexapetalus* could be attributed to the presence of  $\beta$ -caryophyllene oxide.

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**Keywords:** Essential oils, mosquito repellent properties, Annonaceae,  $\beta$ -caryophyllene oxide, cadinol, cubenol.

### INTRODUCTION

The genus *Artabotrys* is among the relatively large genera of the family Annonaceae, consisting of at least 100 species which are distributed in Africa and East Asia (Sagen et al., 2003; Nyandoro, 2014). In Tanzania the genus is represented by *Artabotrys brachypetalus*, *A. collinus*, *A. hexapetalus*, *A. modestus*, *A. monteiroae* *A.*

*rupestris* and *A. stolzii* (Samwel, 2010). Few species of the genus *Artabotrys* have been investigated for their essential oils composition (Menut et al., 1992; Fournier et al., 1997; Garg and Siddiqui, 1999; Phan et al., 2007; Srivastava et al., 2009; Juma, 2009; Dai et al., 2010; Thang et al., 2013). These include *A. lastourvillensis* (Menut et al., 1992), *A. insignis*, *A. pierreanus*, *A. rufus*, *A.*

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DOI : <http://dx.doi.org/10.4314/ijbcs.v8i6.37>

*thomsoni*, *A. venustus* (Fournier et al., 1997), *A. odoratissimus* (Garg and Siddiqui, 1999; Srivastava et al., 2009), *A. hexapetalus* (Phan et al., 2007), *A. modestus* (Juma, 2009), *A. vinhensis* (Dai et al., 2010) and *A. hongkonensis* (Thang et al., 2013). Essential oil investigations from *A. modestus*, a species endemic to Tanzania revealed presence of uncommon constituents, significant amount of oil yield for commercial exploitation and showed moderate mosquito repellence (Juma, 2009). These results prompted us to investigate other unexplored *Artabotrys* species occurring in Tanzania. The overall objective of this study was to establish the chemical composition of essential oils from *A. hexapetalus* and *A. rupestris* and evaluate their mosquito repellent activities against female *Anopheles* mosquito (*An. gambiae*). Thus herein, we report results for *A. hexapetalus* and *A. rupestris*, the former species having essential oil previously reported from flowers only (Phan et al., 2007) while the later which is an endangered species endemic to Tanzania (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009) had not been investigated for its essential oils. The chemical composition of the leaf, stem and root essential oils of *A. hexapetalus* and stem oils of *A. rupestris* and their mosquito repellency are hereby reported for the first time.

## MATERIALS AND METHODS

### Plant materials collection

The leaves and stem bark of *A. hexapetalus* were collected in February 2012 from a thicket at Fukayosi village, Bagamoyo District, in Pwani Region, Tanzania. The stem bark of *A. rupestris* was collected in April 2012 from Mhangala Forest Reserve, Mufindi District, in Iringa Region, Tanzania. All plant species were identified in the field and confirmed at the Herbarium of the Botany Department, University of Dar es Salaam

where voucher specimens are deposited, with reference number FMM 3584 and FMM 3585 for *A. hexapetalus* and *A. rupestris*, respectively.

### Hydrodistillation of essential oils and other general experimental procedure

The plant parts (leaves, stem and root barks) were dried at room temperature in the shade and chopped into small pieces using a grinder. Fractional hydrodistillation was carried out to extract oils from the plant parts using a Clevenger-type water steam distillation apparatus and the process continued until all the essential oils were extracted from the plant materials. The extraction procedure was carried out as previously described by Innocent et al. (2010) with modifications involving collections of fractions. The oil was collected at interval of 50 min in order to get three different fractions. This was controlled by checking the oil droplets out from the condensate tube through the glass tube of the Clevenger apparatus and when there were no more oil droplets coming out, the distillation was stopped. The oil was freed from dissolved and suspended water by partitioning with petroleum ether and left overnight to allow the solvent to evaporate. The oil was then treated with anhydrous sodium sulphate to remove water content. This served to prevent subsequent hydrolysis of esters and other hydrolysable constituents of the oil, hence helping to preserve its odor and properties. The oil yields were calculated based on dried weight of the plant material then stored in a refrigerator at 4 °C for further analysis and mosquito repellent test.

### Determination of chemical compositions of essential oils

The chemical compositions of the essential oils constituents was established by GC-MS performed on a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph. Electron ionization

was done at 70 eV with an ion source temperature at 200 °C. An HP-5 column (30 m long and internal diameter of 0.25 mm with film thickness of 0.25 µm) was used. Oven temperature was programmed from 60 °C (5 min) to 250 °C at 5 °C/min, 5 min hold. Helium (99.999%) was used as the carrier gas and in some cases where separation of the peaks was not good the temperature program and the programs rate were adjusted. Each sample was dissolved in hexane to give a 1% w/v solution; 0.2 µL of the oil sample were injected into the GC-MS equipment at a temperature of 250 °C from which the GC chromatograms and MS spectra were obtained. The GC chromatograms were used for the determination of the abundance of the individual constituents while MS spectra were used for identification of the constituents through matching the mass spectra of the constituents to those recorded in the Wiley 275 and NIST Mass Spectral Library with the associated database and available literature.

#### **Mosquito repellency assays**

Mosquito repellency assay procedure as described by Innocent et al. (2010) was adopted. The mosquito repellency assays were carried out at Amani Research Centre of the National Institute for Medical Research (NIMR) Muheza District, Tanga Region, in Tanzania. All the tests for repellency were conducted on 5 to 7 days old mosquitoes that were fed on 10% glucose solution before the experiment. The mosquitoes were starved for 18 hours before the repellency experiment. The bioassays were conducted in a dark room at 26-30 °C and 80% humidity in order to mimic the host feeding condition for the female *An. gambiae* s.s. mosquitoes. Human volunteers were used in the repellency test. The volunteers who were not allergic to mosquito bites and did not apply any lotion, perfumes and oils on the tested arm on the day of the bioassays were chosen. Different concentrations for test solution of the oil were prepared by dissolving 1 part of sample in 9 parts of analytical grade acetone followed by

ten-fold dilution to obtain the subsequent concentrations ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  g/mL, respectively). Then each concentration was divided into three portions in order to get an average number of mosquito bites. Each solution (0.5 mL) was spread on the right forearm (average area 696.6 cm<sup>2</sup>) of a volunteer from the wrist to the elbow. Gloves were used to cover the excluded areas of the arm. Acetone (0.5 mL) was spread on the left forearm to act as control. The control arm was introduced into the cage (30 cm x 30 cm x 30 cm by volume), containing 50 test mosquitoes and were exposed for 3 min. The mosquitoes that had landed on that arm during the test duration were recorded. The treated arm was then introduced into the cage and kept for 3 min. The number of mosquitoes that landed on the treated arm was also recorded. The landed mosquitoes were shaken off the arm before sucking blood. The arms were washed with soap, rinsed with water and allowed to dry before application of another dose. Each concentration was analysed by using a fresh batch of mosquitoes.

#### **Statistical analysis**

The percentage protective efficacy (PE) was estimated by Abbot Formula as:  $P.E = (ZC - ZT) / ZT \times 100$ , where ZC and ZT are the mean numbers of mosquito that landed on control and treated arms, respectively (Innocent et al., 2010; SAS Institute, 2000). The data were subjected to analysis of variance (ANOVA) and the mean percentage protection compared using Students-Newman-Keuls (SNK) of SAS package. Probit analysis to compute repellency concentration at 50% protection ( $RC_{50}$ ) was done using the Lackfit Inversal procedure of the SAS programme (SAS Institute, 2000). A P value < 0.05 was considered statistically significant.

## **RESULTS**

#### **Chemical compositions of the essential oils**

GC-MS analysis of the essential oil from the stem bark of *A. hexapetalus* indicated high amount of sesquiterpenes of

approximately 86.41%, 69.2% and 77.78% for the first, second and third fractions, respectively (Table 1). The constituents which were represented in the respective fractions in a significant amount were  $\beta$ -caryophyllene oxide (45.62%, 48.78% and 67.98%), and  $\zeta$ -elemene (5.57%, 7.30% and 3.93%). Other constituents were 1,3,3-trimethyl-2-(2-methylcyclopropyl)-cyclohexene (8.62%), 4 $\alpha$ ,8-dimethyl-2-(1-methylethylidene)-naphthalenone (7.97%) obtained from the first fraction (Table 1) while isoaromadendrene epoxide (7.52%), (-)-spathulenol (7.30%) and tricyclo[4.2.2.2(2,5)]dodecan-3-ol (7.68%) were the main compounds of the second fraction (Table 1). The third fraction further contained 7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (13.01%), and 1-methyl-6-(3-methylbut-1,3-dienyl)-7-oxabicyclo[7.1.0]heptanes (5.20%) in moderate amount (Table 1). Other minor components are also included in Table 1.

The GC-MS analysis of the essential oil from leaves of *A. hexapetalus* indicated the first and second fractions to consist of twelve and six compounds, respectively (Table 2). Of the constituents present in the fractions,  $\beta$ -caryophyllene oxide was identified as the major component of the first and second fractions at 39.65% and 37.46%, respectively. The two oil fractions indicated similar other constituents with significant composition of the cadinol (10.89% and 27.52%) and 11-hexadecynal (8.41% and 8.71%). The third fraction of the oil indicated small number of constituents with lilac alcohol and (*E*)-tetra-dec-10-en-1-ol being the major components at 43.82% and 44.45%, respectively (Table 2). Furthermore, phytol was identified in the second and third fractions of the oil (16.76 and 7.18%, respectively).

GC-MS analysis of the essential oil from the stem bark of *A. rupestris* indicated different numbers of oil component for all

three fractions (Table 3), with 6-(*p*-tolyl)-2-methyl-2-heptanol being the predominant compound in the first and second fractions (44.49% and 42.25%, respectively) while (-)-spathulenol (43.60%) was the main constituent of the third fraction. The three fractions also contained high amount of alcoholic and unsaturated sesquiterpenes (58%, 56.25%, 60%, respectively) with 7,11,15-trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene (25.36%),  $\beta$ -santalol (8.20%) and (-)-spathulenol (6.00%) being the other significant constituents from the first fraction. Other constituents of the second fraction were 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol (15.5%), 1,5,5-trimethyl-2-methylene-bicyclo[4.1.0]heptanes-7-methanol (9.97%) and (-)-spathulenol (6.00%) while 3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene (11.46%), 1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene (22.11%) were the other components of the third fraction.

#### Mosquito repellency of essential oils

The dose-response repellency of the extracted essential oils was performed at different concentrations in triplicate against female *Anopheles* mosquito (*An. gambiae*) by applying 1 mL of each sample concentration on the fore arms. The results showing the effect of essential oil treatment of various samples from the two *Artabotrys* species as compared to citronellal (a standard repellent used) gave raw data which were statistically analysed as percentage efficiency. The percentage efficiency were further computed to obtain repellency concentration that repels 50% of the test organism ( $RC_{50}$ ) values using the excel spread sheet and SAS computer programs (SAS Institute, 2000), the results of which are summarized in Table 4 for ten different samples.

**Table 1:** Chemical compositions of essential oil from the stem bark of *Artabotrys hexapetalus*.

| Name of Compounds  | % Composition  |                 |                |
|--|----------------|-----------------|----------------|
|  | First fraction | Second fraction | Third fraction |
| Elemene  | 5.57           | 7.30            | 3.93           |
| 4a,8a-Dimethyl-2-(1 <i>H</i> )-naphthalenone                     | 1.54           | 0               | 0              |
| 2,6,10-Dodecatrien-1-ol  | 1.83           | 0               | 0              |
| Caryophyllene  | 3.04           | 0               | 0              |
| 4a,8a-Dimethyl-2-(1-methylethylidene)-naphthalene                | 7.97           | 0               | 0              |
| $\beta$ -Caryophyllene oxide                                     | 45.62          | 48.78           | 67.98          |
| 1,3,3-Trimethyl-2-(2methyl-cyclopropyl)-cyclohexene              | 8.62           | 3.15            | 0              |
| 12-Oxabicyclo(9,10)dodeca-3,7-diene                              | 6.99           | 0               | 5.20           |
| Isoaromadendrene epoxide   | 4.68           | 7.52            | 0              |
| (-)-Spathulenol  | 1.92           | 7.30            | 0              |
| Cadinol  | 0.92           | 0               | 0              |
| Globulol   | 0.49           | 0               | 0              |
| Lendrene oxide   | 0.69           | 0               | 0              |
| Tricyclo(7,16)triacontane  | 3.35           | 0               | 0              |
| 1-Ethyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane            | 0              | 2.22            | 0              |
| 7-Methyl-4-methylene-1-(1-methylethyl)-naphthalene               | 0              | 2.27            | 13.01          |
| Cubenol  | 4.83           | 0               | 0              |
| Tricyclo[4.2.2.2(2,5)]dodecan-3-ol                               | 0              | 7.68            | 0              |
| <i>Cis</i> -2-isopropylbicyclo[4.3.0]non-3-en-8-one              | 0              | 1.99            | 0              |
| <i>Cis</i> -Z- $\alpha$ -Bisabolene epoxide                      | 0              | 3.06            | 0              |
| 1,1,4,7-tetramethyl-1 <i>H</i> -Cycloprop[e]azulen-4-ol          | 0              | 4.24            | 2.52           |
| 1,4a-Dimethyl-7-(1-methylethylidene)-1-naphthalenol              | 0              | 2.75            | 0              |
| Pentadecanoic acid   | 0              | 3.71            | 0              |
| Santalol   | 0              | 0               | 3.65           |
| Longiverbenone   | 0              | 0               | 1.68           |
| 11-Hexadecynal   | 0              | 0               | 2.01           |
| 1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptanes | 0              | 0               | 14.42          |

## DISCUSSION

From the essential oils composition analysis (Tables 1-3), it can be realized that the two Tanzanian *Artabotrys* species investigated consisted of five compounds that were common to all. These are  $\beta$ -caryophyllene oxide, cadinol, cubenol, (-)-spathulenol and 7-methyl-4-methylene-1-(1-methylethyl)-naphthalene.  $\beta$ -Caryophyllene oxide was present in significant amount in both species while cadinol and cubenol were present in relatively small amounts compared to the other compounds. The five identified compounds common to the two species are also reported to be found in the essential oil of other *Artabotrys* species and some of the

Annonaceaeous plant species. Thus,  $\beta$ -caryophyllene oxide and cadinol had been reported from the fruits and leaves of *A. ordratissimus*, respectively (Garg and Siddiqui, 1999; Srivastava et al., 2009).  $\beta$ -Caryophyllene oxide, cadinol and cubenol have also been found in the oil of stem bark of *Artabotrys modestus* (Juma, 2009). Spathulenol is reported to be the main constituents of the leaves oil of *Uvaria rufa* (Juma, 2009). Furthermore,  $\beta$ -caryophyllene oxide has also been reported in essential oil from the leaves of *Asteranthe asterias* (Juma, 2009) and as a constituent of many other plant species essential oils.

**Table 2:** Chemical compositions of essential oil from the leaves of *Artabotrys hexapetalus*.

| Name of Compounds  | %Composition   |                 |                |
|--|----------------|-----------------|----------------|
|  | First fraction | Second fraction | Third fraction |
| 3,7-Dimethyl-2,6-octadiene   | 2.75           | 0               | 0              |
| Lilac alcohol  | 4.76           | 0               | 0              |
| Lilac alcohol formate  | 1.95           | 0               | 0              |
| 1,3,3-Trimethyl-2-oxabicyclo-octan-6-ol acetate                    | 1.49           | 0               | 0              |
| 7-Methyl-4-methylene-1-(1-methylethyl)-naphthalene                 | 2.88           | 0               | 0              |
| 1-[3-Methyl-3-(4-methyl-3-pentenyl)]-ethanone                      | 5.53           | 0               | 0              |
| $\beta$ -Caryophyllene oxide                                       | 39.65          | 37.46           | 0              |
| 1,5,5,8-Tetramethyl-1,2-oxabicyclododeca-3,7-diene                 | 5.03           | 0               | 0              |
| Cubenol  | 11.59          | 0               | 0              |
| Cadinol  | 10.89          | 27.52           | 0              |
| 11-Hexadecynal   | 8.41           | 0               | 0              |
| Isoaromadendrene epoxide   | 5.08           | 0               | 0              |
| 2-(5-Ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-5-hepten-3-one | 0              | 1.86            | 0              |
| 1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene           | 0              | 4.843           | 0              |
| Phytol   | 0              | 16.76           | 7.18           |
| Lilac alcohol  | 0              | 0               | 43.82          |
| ( <i>E</i> )-Tetradec-10-en-1-ol                                   | 0              | 8.71            | 44.45          |
| Hexadecanoic acid  | 0              | 0               | 4.53           |

**Table 3:** Chemical compositions of essential oil from the stem bark of *Artabotrys rupestris*.

| Name of Compounds   | %Composition   |                 |                |
|---|----------------|-----------------|----------------|
|   | First fraction | Second fraction | Third fraction |
| $\alpha$ , $\alpha$ ,4-Trimethyl-benzenemethanol  | 0.29           | 0               | 0              |
| 7,11-Dimethyl-3-methylene-1,6,10-dodecatriene   | 2.20           | 0               | 0              |
| 7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene                               | 25.36          | 0               | 0              |
| Santanol  | 8.20           | 0               | 2.59           |
| Decahydro- $\alpha$ , $\alpha$ ,4 $\alpha$ -trimethyl-8-methylene-2-naphthalenemethanol | 4.35           | 0               | 0              |
| Toluic acid   | 1.39           | 0               | 0              |
| 6-( <i>p</i> -Tolyl)-2-methyl-2-heptanol  | 44.49          | 0               | 0              |
| Methyl-5,11,14,17-eicosatetraenote  | 3.22           | 0               | 0              |
| $\alpha$ -Cadinol   | 1.13           | 3.65            | 0              |
| 2-Methyl-2-(4-methyl-3-pentenyl)-cyclopropane-carboxaldehyde                            | 3.10           | 0               | 5.46           |
| 2,2,7,7-Tetramethyltricyclo(1,6)undec-4-en-3-one  | 0.26           | 0               | 0              |
| (-)-Spathulenol   | 6.00           | 0               | 43.60          |
| 2,6,6-Trimethyl-2,4-cycloheptadien-1-one  | 0              | 0.11            | 0              |
| 2,6-dimethyl-6-(4-methyl-3-pentanyl)-bicyclo[3.1.1]hept-2-ene                           | 0              | 0.45            | 0              |
| 7,11-Dimethyl-3-methylene-1,6,10-dodecatriene   | 0              | 0.74            | 3.58           |
| 1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene                                 | 0              | 4.01            | 8.49           |
| 3-(1,5-Dimethyl-4-hexenyl)-6-methylene-cyclohexene                                      | 0              | 4.56            | 11.46          |
| 1,5,5-trimethyl-2-methylene-bicyclo[4.1.0]heptanes-7-methanol                           | 0              | 9.97            | 0              |

|  |   |       |       |
|--|---|-------|-------|
| 7,11-Dimethyl-3-methylene-1,6,10-dodecatriene                              | 0 | 0.74  | 3.58  |
| 1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene                    | 0 | 4.01  | 8.49  |
| 3-(1,5-Dimethyl-4-hexenyl)-6-methylene-cyclohexene                         | 0 | 4.56  | 11.46 |
| 1,5,5-trimethyl-2-methylene-bicyclo[4.1.0]heptanes-7-methanol              | 0 | 9.97  | 0     |
| $\beta$ -Caryophyllene oxide   | 0 | 6.00  | 0     |
| Ledene oxide(ii)   | 0 | 1.31  | 0     |
| (E,E)-7,11,15-trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene            | 0 | 1.51  | 0     |
| 3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol                                   | 0 | 15.50 | 0     |
| 4 $\alpha$ ,8,8-Trimethyl-octahydro-benzo(c)-cyclopropa(d)-pyran-2,4-dione | 0 | 0.62  | 0     |
| Megastigma-4,6(E)8,(Z)-triene  | 0 | 4.27  | 0     |
| 1,7-dimethyl-4-(1-methylethyl)-spiro[4.5]dec-6-ene-8-one                   | 0 | 1.89  | 0     |
| 1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene 1                              | 0 | 0     | 22.11 |
| 1,3-Diisopropenyl-6-methyl-cyclohexene                                     | 0 | 0     | 2.70  |

**Table 4:** Repellent efficacy of the essential oils from *Artabotry rupestris* and *A. hexapetalus*.

| Repellent   | Percentage Repellency/Concentration (mg/mL) |            |            |            | RC <sub>50</sub> (mg/cm <sup>2</sup> )<br>× 10 <sup>-4</sup> |
|-------------|---|------------|------------|------------|--|
|             | 0.0001                                      | 0.001      | 0.01       | 0.1        |  |
| Citronellal | 96.7 ±1.3                                   | 98.0 ±1.2  | 98.7 ±0.7  | 90.7 ±6.4  | 0.041(0.0,20)  |
| ARS 1.1     | 90.0 ±2.3                                   | 88.0 ±4.2  | 86.7 ±5.5  | 86.0 ±1.2  | 1.854(0.0,60)  |
| ARS 1.2     | 74.0 ±7.6                                   | 72.7 ±7.4  | 77.3 ±5.5  | 62.0 ±4.2  | 1.032(0.0,90)  |
| ARS 1.3     | 18.0 ±8.1                                   | 11.3 ±5.9  | 13.3 ±7.7  | 18.7 ±6.4  | 48.139(10,1050)  |
| AHL 1.1     | 56.0 ±3.5                                   | 66.0 ±3.5  | 74.0 ±4.2  | 57.3 ±10.9 | 0.181(0.0,20)  |
| AHL 1.2     | 89.3 ±1.8                                   | 92.7 ±2.4  | 92.0 ±2.3  | 90.0 ±4.0  | 0.274(0.0,280)   |
| AHL 1.3     | 46.7 ±11.4                                  | 42.0 ±12.2 | 32.0 ±12.1 | 53.3 ±8.8  | 13.802(0.0,310)  |
| AHS 1.1     | 72.0 ±4.6                                   | 69.3 ±5.8  | 75.3 ±4.4  | 67.3 ±5.2  | 18.943(0.0,450)  |
| AHS 1.2     | 96.0 ±0.0                                   | 89.3 ±2.4  | 89.3 ±2.4  | 78.5 ±4.9  | 74.747(10,1380)  |
| AHS 1.3     | 94.7 ±1.3                                   | 96.7 ±0.7  | 84.7 ±5.7  | 89.3 ±2.9  | 13.802(0.0,310)  |

ARS = *Artabotry rupestris* stem barks oil sample, AHL = *Artabotry hexapetalus* leaves oil sample, AHS = *Artabotry hexapetalus* stem barks oil sample; sample: 1.1, 1.2, 1.3 = first, second and third fractions, respectively. Values in parentheses represent lower and upper confidence limits at 95%; P < 0.05.

The mosquito repellency results indicated significant difference in activities among the tested samples including standard citronellal (Table 4). Essential oils from the stem bark of *A. rupestris* showed different RC<sub>50</sub> values for the first, second and third fractions marked  $1.854 \times 10^{-4}$  mg/cm<sup>2</sup>,  $1.032 \times 10^{-4}$  mg/cm<sup>2</sup> and  $4.8139 \times 10^{-3}$  mg/cm<sup>2</sup>, respectively. The first two fractions showed high repellent activity compared to the third. The difference in repellency could be due to the difference in oil composition, the activity of the first and second oil fractions being attributed to the same major constituents which is (6-Tolyl)-2-methyl-2-heptanol

whereas the third fraction contained (-)-spathulenol as the major component of the oil which indicated higher RC<sub>50</sub> value (RC<sub>50</sub> =  $4.8139 \times 10^{-3}$  mg/cm<sup>2</sup>) hence less active. Nevertheless, the three fractions indicated low mosquito repellent activity compared to standard citronellal (RC<sub>50</sub> =  $4.1 \times 10^{-6}$  mg/cm<sup>2</sup>).

The first, second and third fractions of *A. hexapetalus* leaves essential oil showed variations of RC<sub>50</sub> value, i.e.  $1.81 \times 10^{-5}$  mg/cm<sup>2</sup>,  $2.74 \times 10^{-5}$  mg/cm<sup>2</sup> and  $1.3802 \times 10^{-3}$  mg/cm<sup>2</sup>, respectively. The activity trend of the first and the second oil fraction were nearly the same but substantially different from the activity of the third fraction. Thus, the activity

of first and second fractions could be attributed to the presence of high amount of  $\beta$ -caryophyllene oxide, which was not present in the third fraction, but instead had high amount of 11-hexadecyn-1-ol. The mosquito repellency efficiency of the first and second fractions were the highest of all tested samples and closer to the activity of the citronellal oil, which was used as standard reference (Table 4).

The essential oil from the stem bark of *A. hexapetalus* when evaluated for the mosquito repellency indicated low activity. The third fraction of the oil indicated low value at  $RC_{50}$   $1.3802 \times 10^{-3}$  mg/cm<sup>2</sup>, compared to the first and second fractions which showed higher values at  $RC_{50}$   $1.8943 \times 10^{-3}$  mg/cm<sup>2</sup> and  $7.4747 \times 10^{-3}$  mg/cm<sup>2</sup>, respectively, indicating low activity. The three fractions from stem bark of *A. hexapetalus* registered lower activity compared to citronellal (Table 4).

The chemical composition analysis of the essential oils constituents has indicated the sesquiterpene  $\beta$ -caryophyllene oxide to be present in almost all parts of the *Artabotrys* species investigated; with high percentage composition in the first and second fractions of leaves essential oil of *A. hexapetalus*. Consequently, these fractions were observed to exhibit high repellency activity in comparison to other fractions. Thus, the observed high mosquito repellent activity of these essential oil fractions could be attributed to the presence of  $\beta$ -caryophyllene oxide. The results from these investigations have shown great potential of essential oil bearing *Artabotrys* species for further exploration for their repellent properties against mosquitoes and other insects.

#### ACKNOWLEDGEMENTS

The Belgian Technical Cooperation (BTC) is highly appreciated for financial support to accomplish the reported research project. Sincere gratitude to Mr. F.M. Mbago, the plant taxonomist at Herbarium of the Botany Department, University of Dar es Salaam for identification and collection of the plant species investigated. Mr. E. Gwae and

Mr. F. Kway of the Government Chemist Laboratory Agency (GCLA) are acknowledged for their assistance during essential oil composition analysis. The authors are also grateful to Mr. S.F. Magogo, Mr. F.A. Masawe and Dr. P.K. Tungu of Amani Malaria Research Centre Ubwari Station for facilitating mosquito repellency bioassay.

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